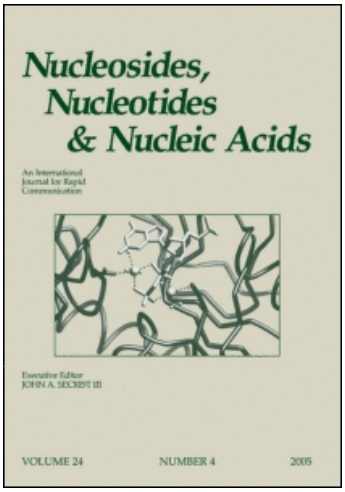

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THE IDENTIFICATION OF 5'-DEOXY-5'-METHYLTHIOGUANOSINE IN HUMAN URINE BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Abstract. 5'-Deoxy-5'-methylthioguanosine (MTG) has been identified in human urine by combined gas chromatography/mass spectrometry. Preliminary identification of MTG in the urine of a lung cancer patient was based upon mass spectral comparisons of the trimethylsilylated (TMS) urinary component to both MTA-$(TMS)_3$ and guanosine-$(TMS)_5$. Structural confirmation was obtained by comparing the mass spectral and chromatographic characteristics of authentic MTG to those of the urinary component.

Introduction

5'-Deoxy-5'-methylthioadenosine (**1**; MTA) was first characterized as a naturally occurring nucleoside in biological systems during the early part of this century[1,2]. Subsequent studies have revealed that MTA is a fundamental component of the complex system responsible for cell growth and proliferation[3,4]. Consequently, the biochemical significance of **1** has been the focus of considerable interest[5,6]; for example, inhibitors of the enzymes involved in MTA metabolism have been examined as potential chemotherapeutic agents[7-9].

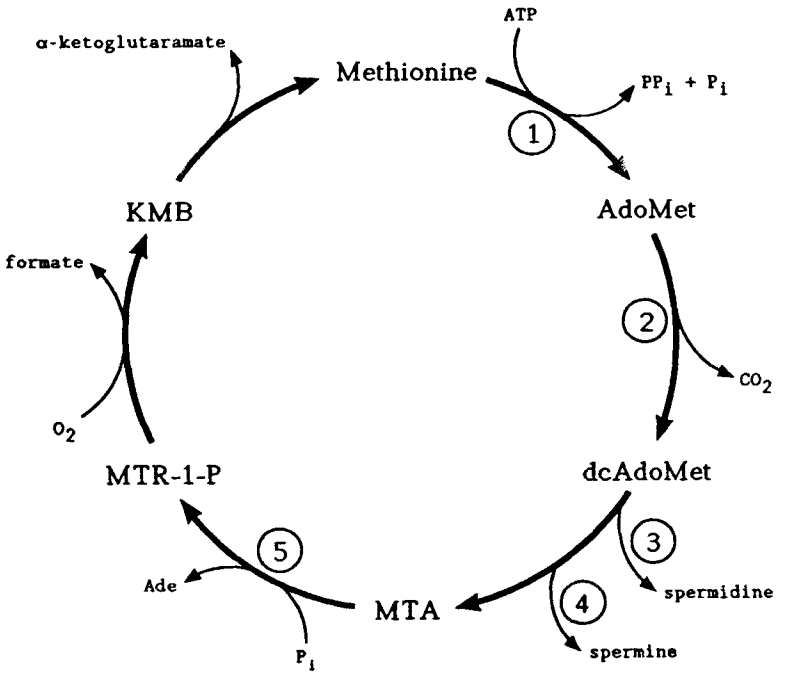


FIG 1. Methionine recycling pathway. Enzymes involved are: **1**, S-adenosylmethionine synthase; **2**, S-adenosylmethionine decarboxylase; **3**, spermidine synthase; **4**, spermine synthase; **5**, methylthioadenosine phosphorylase.

Figure 1 summarizes what is currently known concerning the role of MTA in biological systems[3,4]. Derived primarily from S-adenosylmethionine (AdoMet) or decarboxylated AdoMet (dcAdoMet) as a product in the biosynthesis of the polyamines spermidine and spermine, MTA is converted to Ade and 5-methylthioribose-1-phosphate (MTR-1-P) by methylthioadenosine phosphorylase (MTAPase)[10]. MTAPase is responsible for regulating cellular levels of MTA [if allowed to accumulate, MTA has been shown to produce profound metabolic effects[8,11]]. By generating Ade, MTAPase indirectly maintains adenine nucleotide pools, which can in turn be recycled into the polyamine biosynthetic pathway. The remain-

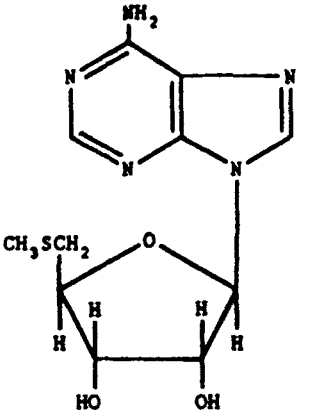
der of the cycle, i.e. conversion of MTR-1-P to methionine, has not been fully elucidated but is believed to involve the conversion of MTR-1-P to formate and α-ketomethylthiobutyric acid (KMB) through a series of oxidative steps[12]; KMB then undergoes a glutamine-dependent transamination to give methionine and α-ketoglutaramate[13-15].

The formation of methionine from MTR-1-P has been reported in rat liver homogenates[12] and in non-mammalian systems[16], but not all human cell lines appear capable of utilizing MTA as a source of methionine[17]. For example, the inability of some leukemia cell lines to convert MTA to methionine is attributed to an MTAPase deficiency[13]. However, these cells can use 5'- deoxy-5'-methylthioinosine (MTI; **2**), the deaminated form of MTA, to generate methionine; cleavage of the glycosidic bond of MTI by purine nucleoside phosphorylase (PNP) yields MTR-1-P and hypoxanthine[8]. Although MTI can serve as an alternative source of MTR-1-P in the event of a cellular MTAPase deficiency[13], the biological significance of this pathway is not clear since MTA is not deaminated to MTI to any significant extent in mammalian tissues[4].

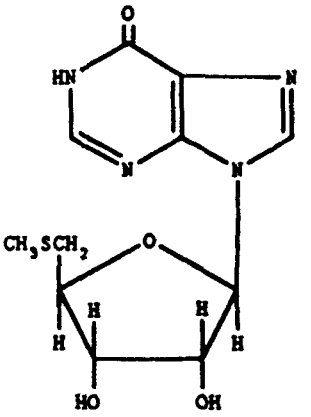
The identification and quantification of MTA in the urine of normal subjects and cancer patients[18] and immunodeficient children[19] was undertaken in an effort to identify "biological markers" in the diagnosis of disease states. The increased polyamine biosynthesis accompanying malignant cell growth led to expectations of elevated MTA levels in the cancer patient urines. However, results indicated no significant differences in urinary MTA excretion between normal and cancer subjects, apparently because of the efficient removal of MTA by MTAPase. In contrast, MTA levels were elevated by greater than three standard deviations (compared to a control group) in the urine of children with severe combined immunodeficiency, although a definitive explanation of these data was not provided.

With this background on the significance of MTA in mind, we report the identification of a novel 5'-methylthioribosyl nucleoside, 5'-deoxy-5'-methylthioguanosine (MTG; **3**), in the

urine of a male subject with advanced lung cancer. A tentative structural assignment of MTG as its trimethylsilyl (TMS) derivative was based on a comparison of the mass spectrum of the urinary component with the mass spectra of MTA-$(TMS)_3$ and guanosine-$(TMS)_5$. Conclusive evidence was obtained by comparing the chromatographic and mass spectral properties of a synthetically prepared reference sample of MTG to the unknown urinary nucleoside. Accurate mass analysis of MTG-$(TMS)_4$ confirmed the identities of the major fragment ions. The analytical and synthetic procedures used in the structural elucidation of MTG are described and its possible biochemical origin is discussed.



1, MTA



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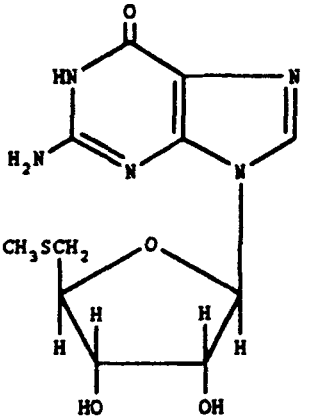
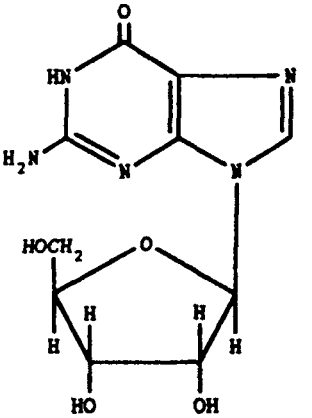


3, MTG



4, G

Experimental

Materials. Silylation grade pyridine and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) were purchased from Pierce Chemical Co. (Rockford, IL). HPLC-grade methanol, ammonium acetate buffer, acetonitrile and formic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). MTA and G were purchased from Sigma Chemical Co. (St. Louis, MO).

Urine Collection and Boronate Gel Affinity Chromatography. A 20-ml aliquot of a 24-hr urine sample obtained from a 75-year-old male with stage IV lung adenocarcinoma was centrifuged to remove particulate matter and stored at -20°C prior to analysis. The boronate gel affinity chromatographic methods used in the isolation of the cis-diol components of the urine samples have been previously described[20,21].

Reversed-Phase HPLC Fractionation of Urinary Nucleosides. The reversed-phase HPLC system consisted of a Shimadzu LC-6A instrument (Shimadzu, Kyoto, Japan) equipped with a SPD-6AV UV- VIS detector (260 nm), two CAPCELL PAK C_{18} columns (250 x 4.6 mm I.D.; 5 μm particle size; Shiseido, Tokyo) connected in series and a CAPCELL PAK C_{18} precolumn (20 x 4.6 mm I.D.; 10-25 μm particle size). The following gradient elution profile was used: initially 2:98 (v/v) 25 mM ammonium acetate buffer (pH 4.5) and acetonitrile, adjusted to 8:92 at 25 min, 30:70 at 35 min, 80:20 at 45 min, and 2:98 at 50 min. The flow rate was 0.8 ml/min with an injection volume of 300 or 350 μl. Approximately forty fractions were collected for each urine sample and the solvent removed by lyophilization.

Derivatization. Each nucleoside fraction was reconstituted in water to a concentration of 1 μg/μl (the amount of material in each fraction was estimated on the basis of its UV absorbance) and a 20-μl aliquot taken to dryness under a stream of nitogen. The TMS derivatives were prepared by adding BSTFA/TMCS (40 μl) in pyridine (10 μl) and heating at 100°C for 1 hr in a closed reaction vessel[22].

Mass Spectrometry. GC/MS analyses were performed using a Varian Model 3400 gas chromatograph coupled to a Finnigan MAT 90 double-focusing instrument (Finnigan MAT, San Jose, CA). Sample introduction was via splitless injection onto a fused silica DB-5 capillary column (30m x 0.25mm; 0.25 μm film thickness; J&W Scientific, Folsom, CA) inserted directly into the ion source. The following GC conditions were used: initial column temperature, 150°C; program rate, 6°C/min; final temperature, 300°C (held for 5 min); helium carrier gas at 10 psi head pressure; injector port and transfer line temperatures, 300°C. The sample injection volume was 0.5 μl. The electron ionization (EI) mass spectrometry conditions were as follows: ionizing energy, 70 eV; emission current, 1.0 mA; source temperature, 250°C; scan range, *m/z* 70 to 1000; scan rate, 0.4 s/decade; resolution, 1000 (10% valley definition). High resolution mass measurements were carried out at a scan rate of 5.0 s/decade and a resolution of 7500, with PFK used for mass calibration.

NMR. ^{1}H-NMR spectra of authentic MTG were obtained on a 300 MHz Varian XL-300 spectrometer (Varian Instruments, Sunnyvale, CA) using perdeuterated dimethylsulfoxide (DMSO-d_6).

UV. UV analyses of the synthetic MTG sample were recorded on a Perkin-Elmer Lambda 7 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) equipped with temperature controlled cell holders set to 25°C.

Elemental Analysis. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA).

Synthesis of MTG. A solution of elemental sodium (50 mg, 2.2 mmol) in anhydrous methanol (30 ml) was cooled to 0°C and saturated with methyl mercaptan for 20 min. 5'-Chloro-5'-deoxyguanosine (302 mg, 1 mmol)[23,24] was added and the mixture allowed to warm to room temperature under argon for 2 h. The mixture was heated to reflux for 24 h., cooled to room temperature, neutralized (pH 7) with 1 N HCl and evaporated to dryness. The residue was dissolved in 5 ml of a 30:70 (v/v) solution of n-propanol/water and applied to a column (5.0 x 90.0 cm) of Biogel P-2 (Bio-Rad Laboratories,

Richmond, CA). The column was washed with the n-propanol/water solution at a flow rate of 2 ml/min. One hundred 50-ml fractions were collected and those containing the desired product, as detected by TLC, were combined and evaporated to dryness. The product was recrystallized from water to afford a white solid (76 mg, 24% yield) having a melting point of >260°C. ^{1}H NMR: δ 10.63 (br. s, 1H, NH), 7.92 (s, 1H, H-8), 6.48 (br. s, 2H, NH_2), 5.69 (d, 1H, H-1', J = 6.0 Hz), 5.46 (d, 1H, 2'-OH, J = 5.1 Hz), 5.25 (br. s, 1H, 3'-OH), 4.56 (m, 1H, H-2'), 4.04 (m, 1H, H-3'), 3.97 (m, 1H, H-4'), 2.84 (dd, 1H, H-5', J = 6.0, 14.0 Hz), 2.74 (dd, 1H, H-5', J = 6.6, 14.0 Hz), 2.07 (s, 3H, CH_3S). UV λ_{max} (ε): pH 7, 252.7 nm (10,850); pH 14 (0.1 N NaOH), 265.4 nm (10,850). Anal. calc. for $C_{11}H_{15}N_5O_4S$: C, 42.17, H, 4.83, N, 22.35; found: C, 42.27, H, 4.80, N, 22.30.

Results and Discussion

The reverse phase HPLC profile of the lung cancer patient urine is depicted in Fig. 2. Tentative identification of the major nucleoside components in the twenty-eight fractions collected was based on a comparison of the retention time and UV spectrum of the urinary fraction to those of reference standards. Subsequent GC/MS analysis generally confirmed the provisional assignments, although several fractions also appeared to contain nucleosides of indeterminate structure.

A portion of the GC/MS reconstructed ion chromatogram (RIC) of fraction 18.3 is shown in Fig. 3. Applying established principles for the interpretation of the EI mass spectra of TMS-derivatized nucleosides[25-27], the presence of three nucleosides was suspected (examination of all spectra for fraction 18.3 indicated no probable nucleosides other than those identified in Fig. 3). The mass spectrum of the first component (retention time 23:41 min; spectrum not shown) displayed peaks related to the molecular ion ($[M]^{+\cdot}$, *m/z* 555; $[M-15]^+$, *m/z* 540), the base (B) series ($[B+116]^+$, *m/z* 322; $[B+100]^+$, *m/z* 306; $[B+74]^+$, *m/z* 280; $[B+30]^+$, *m/z*

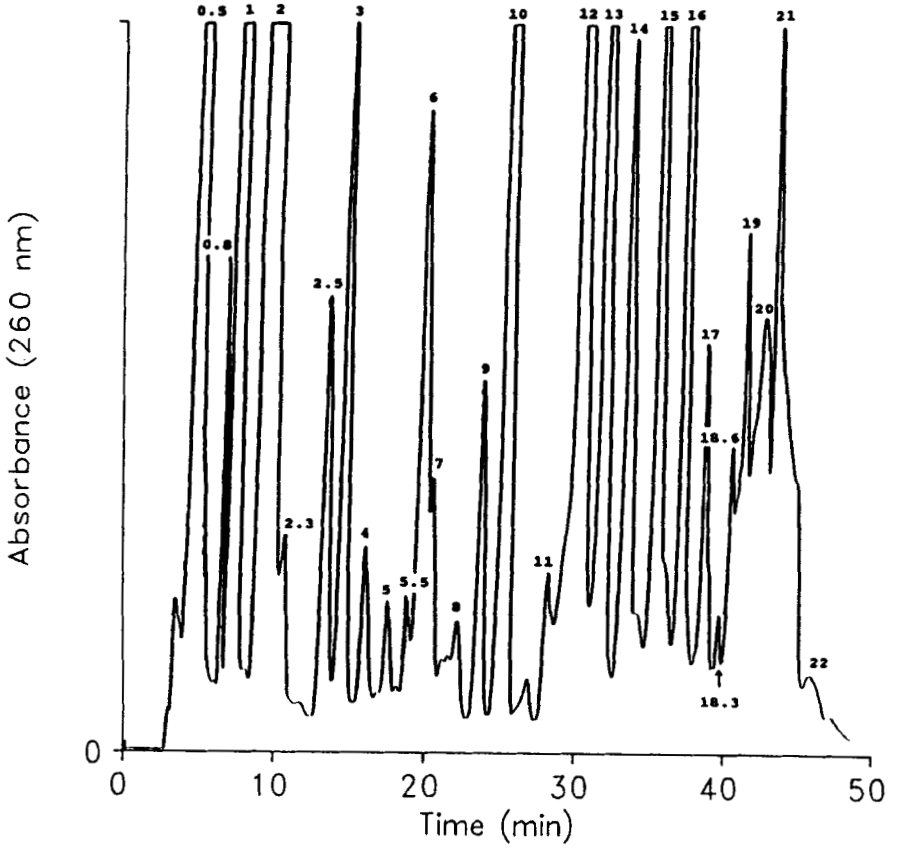


FIG 2. HPLC elution profile of lung cancer patient urine using UV detection at 260 nm.

236 (100% RI); $[B+2]^+$, *m/z* 208; $[B-14]^+$, *m/z* 192) and the sugar (S) series ($[S-1]^{+\cdot}$, *m/z* 348; *m/z* 245, 243, 230, 217 and 103). These data, when compared to the retention time and mass spectrum of the appropriate reference standard (retention time 23:39 min; spectrum not shown), allowed this component to be identified as adenosine-$(TMS)_4$.

The mass spectrum of the component eluting at 25:49 min is given in Fig. 4(a). The molecular weight of this compound can be confidently assigned as 513 u based on the appearance of an ion at *m/z* 513 and an associated $[M-15]^+$ at

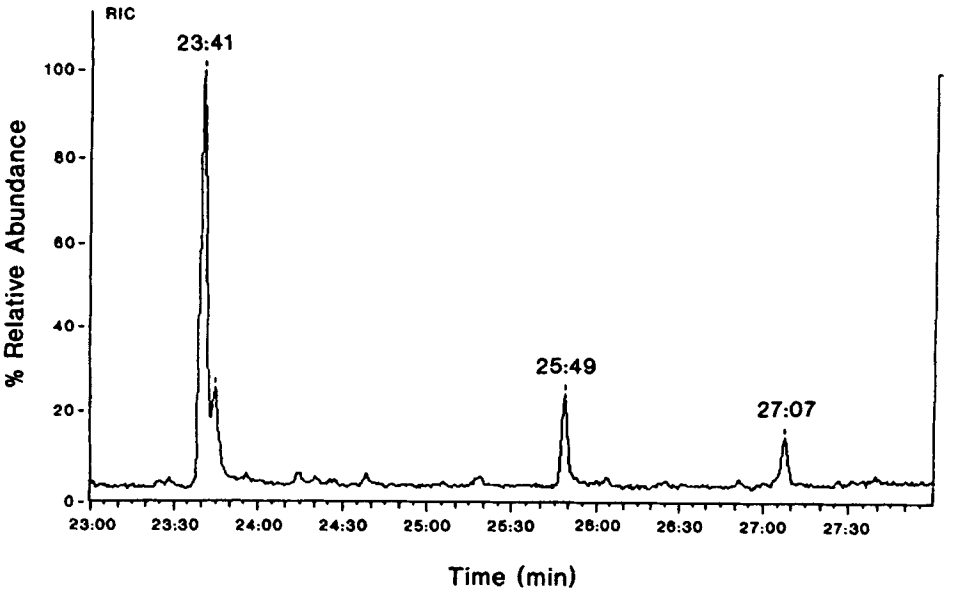


FIG 3. Reconstructed ion chromatogram for urinary nucleoside fraction 18.3 obtained from lung cancer patient.

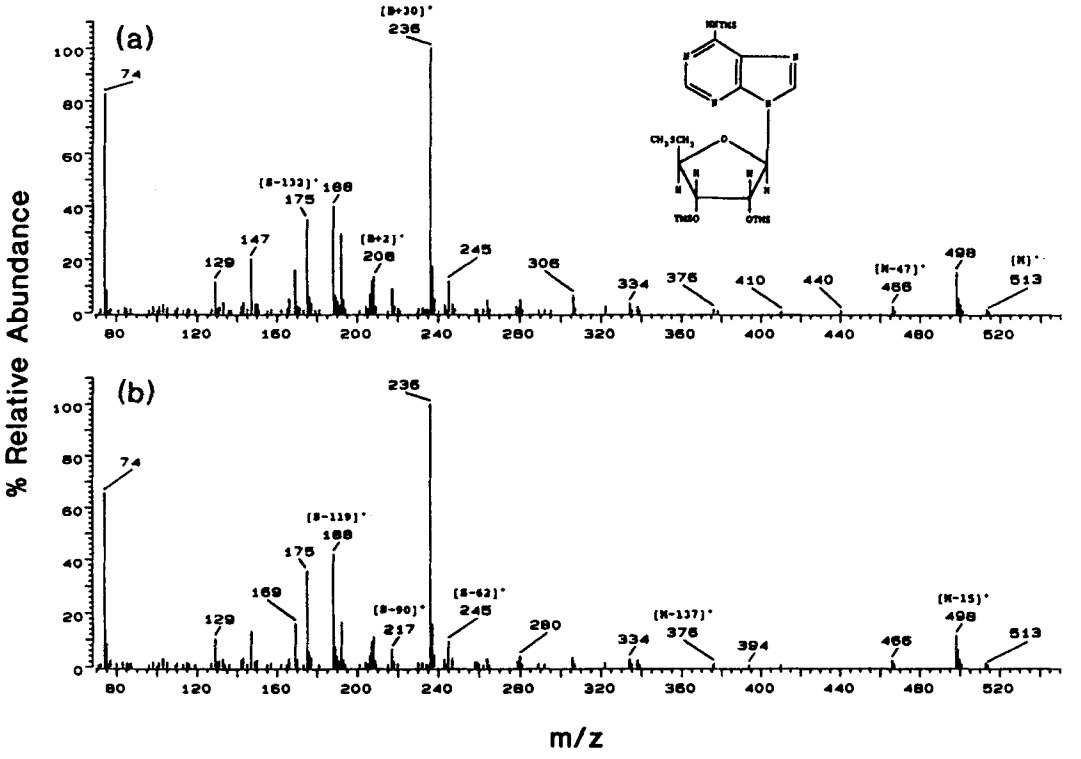


FIG 4. EI mass spectrum of (a) 25:49 min component of fraction 18.3 and (b) reference sample of MTA-$(TMS)_3$ (GC retention time 25:54 min). For detailed assignments, see Table 1.

m/z 498. The fragment ion at *m/z* 466 corresponds to a loss of 47 u from $[M]^{+\cdot}$, a fragmentation process not commonly observed in this compound class[26,27]. However, the expulsion of a methylthio radical ($CH_3S\cdot$), either through cleavage of a side-chain substituent in the base or by fragmentation of a modified sugar, could account for this mass difference. Two factors support the existence of a uniquely modified sugar: (i) the absence of ions normally associated with either ribosyl-, 2'-O-methylribosyl- or 2'-deoxyribosyl-nucleosides (e.g. $[S\text{-}1]^{+\cdot}$, *m/z* 348, 290 and 260 respectively) and (ii) the presence of a B-series of ions encompassing those seen previously in the spectrum of A-$(TMS)_4$: *m/z* 192, 208, 236, 264 ($[B+58]^+$), 280, 306, 334 ($[B+128]^+$) and 338 ($[B+132]^+$). From a putative value of 206 u for B, the sugar moiety is 307 u, corresponding to a shift of 42 u lower than a normal ribose-$(TMS)_3$ and equal to the replacement value of TMSO by CH_3S. The most likely candidate for this adenine/methylthioribose analog appeared to be 5'-deoxy-5'-methylthioadenosine, since MTA had been reported in the urine of cancer patients[18] as discussed earlier. The mass spectrum of a reference sample of MTA-$(TMS)_3$ (Fig. 4(b)) exhibited fragmentation and ion abundances comparable to that of the urinary component (Fig 4(a)), while the similarities in GC retention times (25:49 min versus 25:54 min for authentic MTA-$(TMS)_3$) added further evidence that this component was MTA-$(TMS)_3$.

The mass spectrum of the component eluting at 27:07 min is given in Fig. 5(a). The general similarities in mass spectral features between this component and MTA-$(TMS)_3$ provided a significant aid in establishing identity. For example, in the molecular ion series, $[M]^{+\cdot}$ (*m/z* 601), $[M-15]^+$ (*m/z* 586) and $[M-47]^+$ (*m/z* 554) were easily assigned on the basis of a similar series (*m/z* 513, 498 and 466) in the mass spectrum of MTA-$(TMS)_3$ (Fig. 4(b)). The lack of a recognizable sugar series in Fig. 5(a) also mimicked MTA-$(TMS)_3$ and, coupled with an elimination of 47 u from $[M]^{+\cdot}$, suggested the presense of a methylthioribose (predicted mass

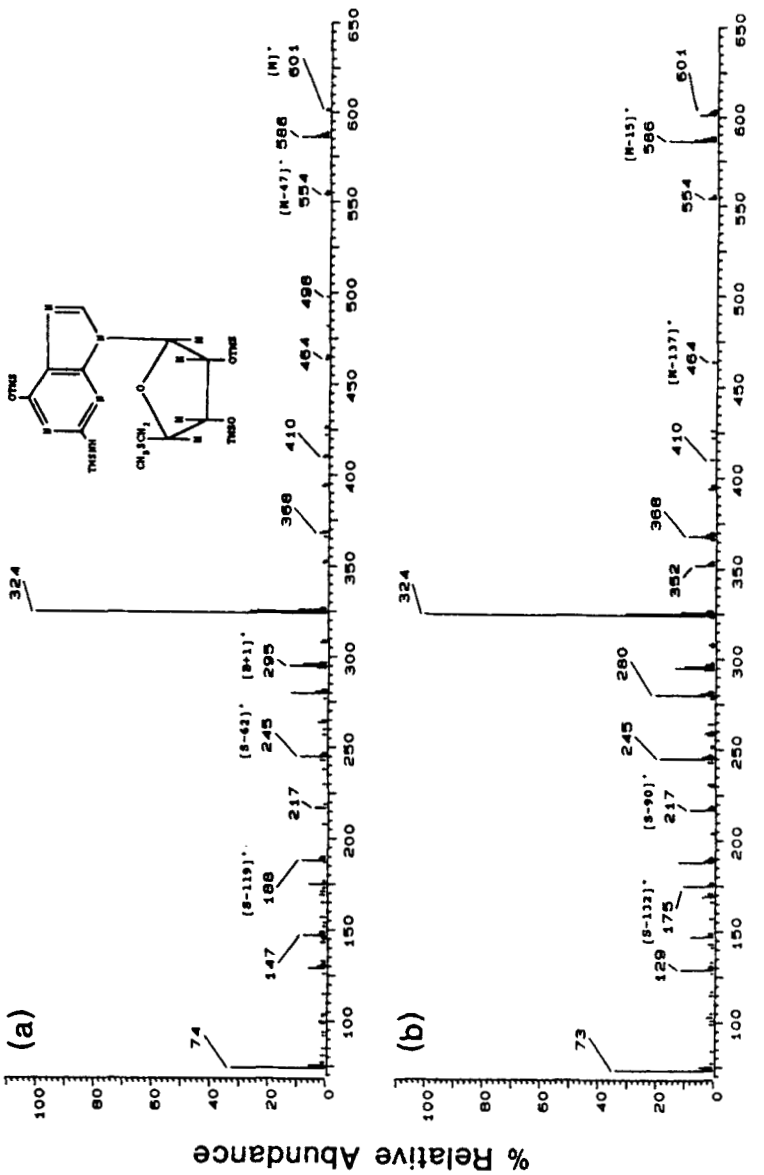


FIG 5. EI mass spectrum of (a) 27:07 min component of fraction 18.3 and (b) reference sample of MTG-$(TMS)_4$ (GC retention time 27:14 min). For detailed assignments, see Table 1.

307 u). Based on this assumption, the aglycone was alloted a weight of 294 u, for which the following base series ions were evident: $[B-14]^+$, *m/z* 280; $[B]^+$, *m/z* 294; $[B+1]^{+\cdot}$, *m/z* 295; $[B+2]^+$, *m/z* 296; $[B+30]^+$, *m/z* 324; $[B+58]^+$, *m/z* 352; $[B+74]^+$, *m/z* 368; $[B+100]^+$, *m/z* 394; $[B+116]^+$, *m/z* 410. Subtraction of 144 u (mass difference for replacement of two TMS groups by two H) from 295 u ($[B+1]^{+\cdot}$) gave 151 u as the weight of the free base. A comparison with the mass spectrum of guanosine-$(TMS)_5$[26] indicated that the B series was consistent with an unsubstituted guanine. Because of these strong parallels with the mass spectra of MTA and G, we deduced that this component might be 5'-deoxy-5'-methylthioguanosine. Figure 5(b) shows the EI mass spectrum of a reference sample of MTG-$(TMS)_4$ having a GC retention time of 27:14 min; the excellent correlation between observed ion abundances and retention times established the structure of this component as MTG-$(TMS)_4$.

Relatively little has been reported concerning the mass spectra of methylthioribosyl nucleosides[18,28]. Table 1 lists the major ions seen in the mass spectra of MTA-$(TMS)_3$, MTG-$(TMS)_4$ and G-$(TMS)_5$; the key ions in differentiating 5'-methylthioriboses from conventional pentoses are found in the sugar ion series and, to a lesser extent, in the molecular ion series.

The elimination of $CH_3S\cdot$ from $[M]^{+\cdot}$ ($[M-47]^+$) is an important indicator of methylthio substitution. A high resolution analysis of MTG-$(TMS)_4$ (Table 2) resulted in an elemental composition of $C_{22}H_{44}N_5O_4Si_4$ for $[M-47]^+$, consistent with the elimination of $CH_3S\cdot$ from $[M]^{+\cdot}$. Careful examination of the mass spectra revealed weakly-abundant high mass ions for both MTA-$(TMS)_3$ (*m/z* 376) and MTG-$(TMS)_4$ (*m/z* 464). Although their low intensities precluded an exact mass determination, these ions are believed to arise through the losses of $CH_3S\cdot$ (47 u) and TMSOH (90 u) from $[M]^{+\cdot}$ (i.e. $[M-137]^+$), analogous to the elimination of TMSOH and CH_3 ($[M-105]^+$) in "conventional" TMS-derivatized nucleosides[26].

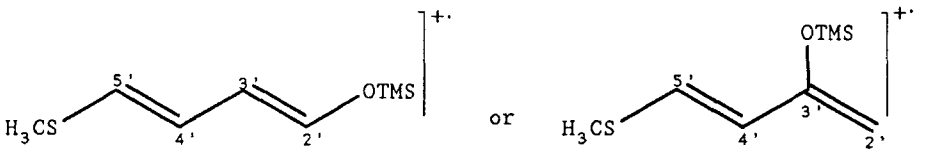
TABLE 1. Structurally Significant Ions in the EI Mass Spectra of MTA-$(TMS)_3$, MTG-$(TMS)_4$ and G-$(TMS)_5$.

MTA-$(TMS)_3$ (m/z)	MTG-$(TMS)_4$ (m/z)	G-$(TMS)_5$ (m/z)	Structural Assignment[a]
	Molecular Ion Series		
513	601	643	$[M]^{+\cdot}$
498	586	628	$[M-15]^+$
466	554	[b]	$[M-47]^+$
376	464	[b]	$[M-137]^+$
	Base Ion Series		
410	498	[b]	$[B+204]^+$
394	482	482	$[B+188]^+$
338	426	426	$[B+132]^+$
334	422	422	$[B+128]^+$
322	410	410	$[B+116]^+$
306	394	394	$[B+100]^+$
280	368	368	$[B+74]^+$
264	352	352	$[B+58]^+$
236	324	324	$[B+30]^+$
208	296	296	$[B+2]^+$
207	295	295	$[B+1]^{+\cdot}$
192	280	280	$[B-14]^+$
	Sugar Ion Series		
[b]	[b]	348	$[S-1]^{+\cdot}$
245	245	[b]	$[S-62]^+$
217	217	259	$[S-90]^+$
[b]	[b]	245	$[S-104]^+$
[b]	[b]	243	$[S-106]^+$
188	188	230	$[S-119]^{+\cdot}$
175	175	217	$[S-132]^+$
[b]	[b]	169	$[S-180]^+$

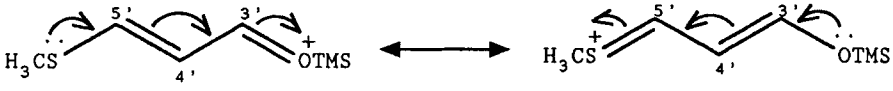
[a] See Ref. 27 for a more detailed explanation of ion structures.
[b] Ion not observed.

Two ions in the sugar series of both MTA-$(TMS)_3$ and MTG-$(TMS)_4$ stand out as unique markers of methylthio substitution: m/z 188 ($[S-119]^{+\cdot}$) and m/z 175 ($[S-132]^+$). In a simple pentose-containing nucleoside such as G-$(TMS)_5$, $[S-119]^{+\cdot}$ and $[S-132]^+$ correspond to the common sugar ions m/z 230 and 217 respectively (see Table 1); based on prev-

ious studies[27,29] and with the aid of exact mass measurements (Table 2), possible structures for *m/z* 188 and 175 include:



m/z 188, $C_8H_{15}SOSi$



m/z 175, $C_7H_{15}SOSi$

As shown in Table 1, $[S-62]^+$ (*m/z* 245) in MTA-$(TMS)_3$ and MTG-$(TMS)_4$ is isobaric with $[S-104]^+$ in G-$(TMS)_5$. Accurate mass measurements in MTG-$(TMS)_4$ also established identical elemental compositions for $[S-62]^+$ (245.1005 u) and $[S-104]^+$ (calc. 245.1029 u) of $C_{10}H_{21}O_3Si_2$. Since $[S-104]^+$ is formed by simple cleavage[27] of the C-4',5' bond in $[S-1]^{+\cdot}$ (i.e. $[S - H - CH_2OTMS]^+$), the analogous fragmentation mechanism is presumed to be in effect for the 5'-methylthioribosyls:

$$\underset{(m/z\ 306)}{[S - H]^{+\cdot}} \rightarrow \underset{(m/z\ 245)}{[S - H - C_2H_5S]^+} + C_2H_5S\cdot$$

The facile elimination of $-CH_2SCH_3$ may provide a rationale for the absense of an appreciable $[S-H]^{+\cdot}$ abundance in the mass spectrum of MTG-$(TMS)_4$.

The elimination of TMSOH from the methylthio sugar ($[S-90]^+$, *m/z* 217) is consistent with the proposed ribosyl mechanism[27] in which the leaving trimethylsilyloxy group originates from either the C-2' or C-3' position. Unfortunately, *m/z* 217 alone is of limited use diagnostically since it is isobaric with the common pentose fragment $C_3H_3O_2(TMS)_2$

TABLE 2. Elemental Compositions and Accurate Mass Measurements of Structurally Significant Ions in the EI Mass Spectrum of MTG-$(TMS)_4$.

m/z (observed)	Error (mmu)[a]	Composition	Assignment
601.2493	-6.7	$C_{23}H_{47}SN_5O_4Si_4$	$[M]^+$
586.2137	+4.3	$C_{22}H_{44}SN_5O_4Si_4$	$[M-15]^+$
554.2431	+2.8	$C_{22}H_{44}N_5O_4Si_4$	$[M-47]^+$
426.1784	+2.1	$C_{16}H_{32}N_5O_3Si_3$	$[B+132]^+$
394.1533	+1.1	$C_{15}H_{28}N_5O_2Si_3$	$[B+100]^+$
368.1736	+1.5	$C_{14}H_{30}N_5OSi_3$	$[B+74]^+$
352.1405	+3.4	$C_{13}H_{26}N_5OSi_3$	$[B+58]^+$
324.1279	+2.8	$C_{12}H_{22}N_5O_2Si_2$	$[B+30]^+$
296.1333	+2.5	$C_{11}H_{22}N_5OSi_2$	$[B+2]^+$
295.1263	+1.7	$C_{11}H_{21}N_5OSi_2$	$[B+1]^+$
280.1028	+1.8	$C_{10}H_{18}N_5OSi_2$	$[B-14]^+$
245.1005	+1.8	$C_{10}H_{21}O_3Si_2$	$[S-62]^+$
217.0670	+4.4	$C_9H_{17}SO_2Si$	$[S-90]^+$
188.0662	+2.5	$C_8H_{16}SOSi$	$[S-119]^+$
175.0607	+0.2	$C_7H_{15}SOSi$	$[S-132]^+$

[a] Error = calculated mass - observed mass; mmu: millimass units.

($[S-132]^+$; Table 1) in low resolution EI mass spectra. However, under high resolution conditions, $[S-90]^+$ (calc. 217.0719 u) in a 5'-methylthioribosyl nucleoside is readily distinguishable from $[S-132]^+$ (calc. 217.1081 u) of the pentose series. It should be noted that while both $[S-62]^+$ and $[S-90]^+$ are suggestive of 5'-methylthio substitution, the key ions *m/z* 188 and 175 must appear concurrently before a definitive assignment can be made.

The biochemical origin and physiological significance of MTG are presently unknown. However, the catabolism of MTA and MTI to MTR-1-P by MTAPase and PNP respectively are well documented processes in the production of methionine in mammalian cells[7,13,30]. We propose a parallel biosynthetic

pathway for MTG as that described earlier for MTI; available MTR-1-P (perhaps derived from MTA) condenses with free cellular guanine to yield MTG, in a reaction catalyzed by PNP. The fact that MTG is a viable substrate for human erythrocyte PNP (Joel V. Tuttle, personal communication) supports this proposal. Ultimately, 5'-deoxy-5'-methylthioguanosine may act as a vehicle for storing methylthioribose[31], later releasing MTR-1-P in the event that MTAPase becomes inhibited or when increased methionine production is required, as in the case of rapid cell proliferation.

Conclusion

5'-Deoxy-5'-methylthioguanosine is the first 5'-methylthioribosyl nucleoside possessing a non-adenine related base to be identified in human body fluids. Studies have indicated that MTG is also excreted in the urine of patients exhibiting clinical symptoms of acquired immunodeficiency syndrome (AIDS) and, to a lesser extent, in the urine of normal individuals (Karl H. Schram et al., unpublished work). The formation of MTG may represent the first example of the two nucleoside phosphorylase enzymes MTAPase and PNP acting in tandem in a mammalian system; earlier work has established the sequential metabolism of 5'-isobutylthioadenosine to 5'-isobutylthioinosine by MTAPase and PNP in cultured human cell lines[31]. Finally, the presence of MTG in human urine suggests that methionine production may not be the sole metabolic fate of MTR-1-P. Efforts to quantitate MTG, MTA and other modified nucleosides are underway, with the hope of determining their relative importance as biological markers of various disease states.

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