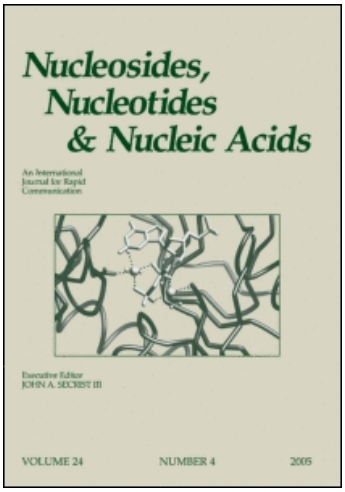

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Girish B. Chhed[a]; Helen B. Patrzyc[a]; Arvind K. Bhargava[b]; Pamela F. Crain[c]; Satinder K. Sethi[c]; James A. McCloskey[ad]; Shib P. Dutta[a]
[a] Departments of Biophysics and Roswell Park Memorial Institute, Buffalo, New York [b] Laboratory Medicine and Roswell Park Memorial Institute, Buffalo, New York [c] Departments of Medicinal Chemistry and Biochemistry, University of Utah, Salt Lake City, Utah [d] Biochemistry, University of Utah, Salt Lake City, Utah

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ISOLATION AND CHARACTERIZATION OF A NOVEL NUCLEOSIDE FROM THE URINES OF CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

Girish B. Chheda[††*], Helen B. Patrzyc[††], Arvind K. Bhargava[§], Pamela F. Crain[†], Satinder K. Sethi[†], James A. McCloskey[†#], and Shib P. Dutta[††]

Departments of Biophysics[††] and Laboratory Medicine[§], Roswell Park Memorial Institute Buffalo, New York 14263
and
Departments of Medicinal Chemistry[†] and Biochemistry[#], University of Utah Salt Lake City, Utah 84112

Abstract

From 24 hour collections of urines of chronic myelogenous leukemia (CML) patients, a novel nucleoside was isolated. It was assigned the structure, 5'-deoxyinosine (I) on the basis of UV, NMR and mass spectrometry and by comparison of the spectral data and HPLC and TLC mobilities with those of the authentic sample. Another nucleoside, 5'-deoxy-5'-methylthioadenosine sulfoxide previously isolated from the urines of immunodeficient children was also found in the urine of a CML patient. Possible origin and significance of both of these nucleosides are discussed.

Introduction

With an objective to look for tumor markers in human body fluids, as well as to study metabolism in man, we have been investigating the modified nucleosides and related substances in the urines of specific groups of cancer patients. Transfer RNA derived urinary nucleosides such as, 1-methylinosine[1,2], N-(purin-6-ylcarbamoyl)-L-threonine riboside (t^6A)[3] and N^2N^2-dimethylguanosine[2], reported earlier, have served as indicators of tumor activity in cancer patients[4-6]. More recently, we have reported the isolation of novel nucleosides, N^7-β-D-ribofuranosylhypoxanthine[7], N^6-succinyladenosine[8] and

*To whom inquiries should be addressed.

N^1-β-D-ribofuranosylpyridin-4-one-3-carboxamide[9] from the urines of cancer patients. These nucleosides originate from sources other than the turnover of nucleic acids. The N^6-succinyladenosine is elevated in the urines of the patients with pancreatic adenocarcinoma and liver adenocarcinoma[10]. These and other related studies[11] suggest that the urinary modified nucleosides can be used as markers for assessing the tumor burden as well as for determining the effectiveness of therapy. This paper deals with isolation and characterization of a novel nucleoside, 5'-deoxyinosine (I, FIG. 1) from the urines of chronic myelogenous leukemia (CML) patients. Also reported herein is the finding of a nucleoside, 5'-deoxy-5'-methylthioadenosine sulfoxide (II, FIG. 1) from the urine of a of CML patient. Mills et al.[12] have recently isolated the latter nucleoside (II) from the urines of immunodeficient children. Nucleoside I was isolated from the 24 hour collections of urines from two patients in 2.1 mg and 0.16 mg amounts, while nucleoside II was isolated in the amount of 0.14 mg from the 24 hour collection from one patient.

MATERIALS AND METHODS

Neutral charcoal (Norit) was purchased from Fisher Scientific Co. Celite 545 was obtained from Johns-Mansville Co. and was washed with 6 N HCl, water, ethanol, and then dried before use. DEAE cellulose (DE-23) and AG-1X-8 formate (200-400 mesh) anion exchange resin were obtained from Whatman and Bio-Rad Labs., respectively. Deuterium oxide (99.96 atom % D) and DMSO-d_6 (99.5 atom % D) were purchased from Aldrich Chemical Co. and Merck Isotopes, respectively. Glass distilled acetonitrile and methanol were obtained from Burdick & Jackson. Deionized distilled water for use in high performance liquid chromatography (HPLC) was prepared in our laboratory. Acid washed papers (grade No. 589) were obtained from Schleicher and Schuell. Sybron polygram silica gel G/U.V. 254 sheets with indicator for thin layer chromatography (TLC) were obtained from Brinkman Scientific Co.

An authentic sample of 5'-deoxyinosine was initially obtained from Prof. R.E. Parks, Brown University, Providence, RI, and subsequently synthesized in our laboratory. The compounds 5'-deoxy-5'-methylthioadenosine and 5'-deoxyadenosine and the enzyme adenosine deaminase (calf intestinal mucosa, type II) were purchased from Sigma Chemical Co. The

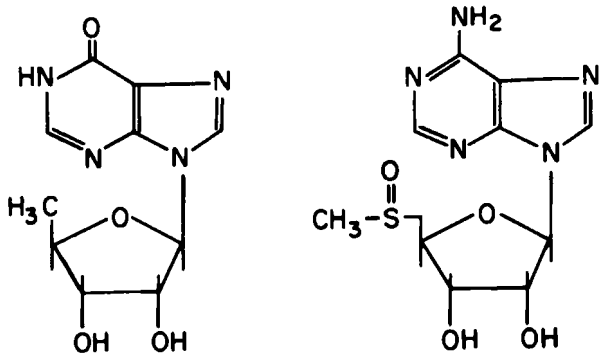

FIG. 1. 5'-deoxyinosine and 5'-deoxy-5'-methylthioadenosine sulfoxide.

compound, 2'-deoxyadenosine was purchased from General Biochemicals, 2'-deoxyinosine and 3'-deoxyadenosine were purchased from Vega Chemicals and 2'-O-methyladenosine was procured from PL Biochemicals. The two latter adenosines were deaminated to corresponding inosines.

Urine Samples: Diagnosis, History and Treatment of Patients

Patient I (J.D.). Urine was collected from a 25 year old male patient, who was diagnosed to have CML at the age of 18. At the time of diagnosis he had marked leukocytosis with WBC of 43000 with 11% blast cells. His bone marrow was hypercellular and he was Philadelphia chromosome positive. During the first two years of the disease he was treated with myleran, Ara C and 6-mercaptopurine. Five years after the diagnosis, the patient became resistant to the above chemotherapy and was treated with hydroxyurea, colcemid and prednisone. The patient had developed splenomegaly during the fifth year and underwent splenectomy. The urine was collected 10 days after the patient had received cytoxan for chemotherapy during the seventh year. The patient survived for a total of seven years and ten months after the diagnosis.

Patient II (R.W.). A thirty-seven year old white male was diagnosed to have CML with basophilic variety. He was found to be Philadelphia chromosome positive. Chromosome cultures showed at least one cell clone containing 49 chromosomes due to an extra chromosome in Group

C. After 2-1/2 years he underwent a therapeutic splenectomy following which he was treated with hydroxyurea, 6-mercaptopurine, 6-thioguanine and prednisone. Urine was collected three weeks after this treatment, 2-3/4 years after the diagnosis. The patient survived for three years and five months after the initial diagnosis.

Patient III (R.M.). The patient had been diagnosed as having CML for about four years and 6 months, when urine was collected. The patient received a bone marrow transplant; he was given Ara C and cytoxan before the transplant and methotrexate after the transplant. A 24 hour urine was collected two weeks after the transplant.

Ultraviolet spectrophotometry. Ultraviolet (UV) spectra were recorded on a Cary 219 spectrophotometer which was zeroed with water using the autobase line feature.

Nuclear Magnetic Resonance (NMR) spectrometry. NMR spectra were determined on a Bruker WP-200 (200 MHz) spectrometer by utilizing the Fourier-transform/quadrature phase detection mode. Sample temperatures were maintained at 30° with a BVT-2000 temperature controller of the WP-200 spectrometer. Unless stated otherwise, the chemical shifts reported here are given in (δ) ppm, and are measured from internal TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-d4). The urinary unknowns and the authentic samples were lyophilized three times from 99.5% D_2O and then dissolved in 99.9% D_2O.

Mass spectrometry (MS) and gas chromatography-mass spectrometry (GC/MS). Initial mass spectra were acquired using a Finnigan 4000; spectral data reported here were obtained on LKB 9000S and Varian MAT 731 instruments with ionizing energy of 70 eV, ion source temperature of 250°C. Low resolution mass spectra were determined with the LKB spectrometer; GC column, 3 ft. 1% OV-17, temp. 170°C. Samples for high resolution measurements by photographic recording (R = 14,000) were introduced to the MAT 731 instrument by direct probe after removal of reagents in the probe vacuum lock. Fast atom bombardment mass spectra of nucleoside II were acquired using the MAT instrument, utilizing an Ion Tech FAB 11N ion source, with a neutral Xe beam of 6 keV energy. For FAB spectral determination, 1.5 µl glycerol was used as the matrix for one µg of the compound.

Chemical derivatization. Trimethylsilylation of the urinary unknown I and the synthetic standards was carried out by heating

approximately 10 µg of vacuum-dried material with N,O-bis(trimethylsilyl)acetamide, trimethylchlorosilane and dimethylformamide (Regis Chemical Co.) (100:1:10) in a sealed melting point capillary tube for 1 hr at 100°C. [$^2H_9$]Trimethylsilyl derivatives were prepared using N,O-bis([$^2H_9$]trimethylsilyl)acetamide and [$^2H_9$]trimethylchlorosilane, purchased from Merck Isotopes.

Chromatography

High Performance Liquid Chromatography (HPLC). HPLC was carried out on Altex Model 330 gradient liquid chromatograph, equipped with an Altex model 420 system controller programmer and an Altex model 153 analytical UV detector with an 8 uL flow cell set at wavelength 254 nm, and with the C-RIA Altex recorder and integrator. An ODS-C_{18} reversed phase column (5 um Ultrasphere analytical 4.6 mm x 25 cm) fitted with a 250 uL loop injector was used for purification and coinjection studies. The following HPLC solvent systems were used for coinjection: (A) Gradient elution, water-methanol; 0 → 20% during 30 min., flow rate 1 ml/min. (B) Isocratic 10% methanol in water; flow rate 1 ml/min. (C) Gradient elution, water-acetonitrile; 0 → 20% during 30 min., flow rate 1 ml/min. (D) Gradient elution, of water-methanol; 5 → 15% during 30 min., flow rate 1 ml/min. For the preparative chromatography, Ultrasphere ODS C_{18} column (10 mm x 25 cm), 5 µm pore size fitted with a 2.0 mL loop injector, Zorbax column ODS C_{18} (22.1 mm x 25 cm) 8 µm pore size fitted with a 5.0 mL loop injector or a Whatman Magnum-9 Partisel 10 ODS-2 C_{18} column (9.4 mm x 50 cm) 10 µm pore size fitted with a 2.0 ml loop injector were used for larger samples.

Chromatography solvents for thin layer chromatography (TLC) and paper chromatography. Ascending TLC, on silica gel G sheets and descending paper chromatography was performed in solvent saturated chambers using the following solvent systems v/v: (a) 2-propanol: water:conc. NH_4OH (7:2:1); (b) ethylacetate:2-ethoxyethanol:16% formic acid (4:1:2) (upper phase); (c) 1-butanol:glacial acetic acid: water (4:1:2); (d) 1-butanol:water:conc. NH_4OH (86:14:5); (e) ethylacetate: propanol:water (4:1:2) (upper phase); (f) propanol:water (60:40); (g) ethylacetate:2-ethoxyethanol:water (4:1:2) (upper phase).

Isolation of the unknown urinary nucleoside I. The 24 hour pooled urine (1210 ml) from a CML patient (J.D.) was adjusted to pH 3 and passed through a column (2.54 x 50 cm) of charcoal-celite (50 g each).

The column was washed with water (3 liters), until the washings were chloride free and the column-bound material was eluted with 3 liters of 2 N NH_4OH in 50% aqueous ethanol. The eluate was concentrated to a small volume (50 ml) and applied to a column (2.54 x 35 cm) of 125 g of AG-1X-8 formate resin. The column was washed with 2.5 liters of water and the wash concentrated to about 50 ml, and then passed through a column of DEAE cellulose equilibrated with 0.14 M boric acid[13]. The latter column was first washed with 3 liters of 0.14 M boric acid and then eluted with 4 liters of 0.70 M boric acid to obtain the nucleoside fraction. The 0.70 M boric acid fraction was evaporated to dryness after repeated addition of methanol (4 x 100 ml). The residue from this fraction was then chromatographed on Whatman 3 MM paper (8 papers, 24 x 57 cm) in solvent b (descending) for 7 hours at room temperature. The bands with the Rf of 0.63 were cut out and eluted with water. The eluate was concentrated to 500 ul and then rechromatographed on Whatman No. 1 (acid washed paper) successively in solvents a, f, c and d. The eluate from the last paper chromatographed in solvent d was lyophilized to give 105 A_{249} units. A portion of this residue (20 A_{249}) was dissolved in 500 ul of water and purified by HPLC on a reversed phase ODS C18 Ultrasphere column using 7% methanol. Fifteen A_{249} units (0.310 mg) of a hitherto unknown nucleoside (I) was obtained. The unknown urinary nucleoside (I) had UV spectra similar to those of inosine with λmax (nm) at 249 (H_2O), 249 (pH 1.0) and 253 (pH 11.0) (Fig. 2a). This material was used for mass spectral and NMR analysis as well as for HPLC coinjection and TLC studies.

Isolation of Nucleoside I by Use of preparative HPLC. The nucleoside I was isolated from the urine of a second CML patient (R.M.) by preparative HPLC instead of paper chromatography. Urine was collected, desalted and fractionated on a DEAE cellulose column in a manner similar to the one described above. The 0.7 M boric acid fraction was dissolved in 20 ml of water, filtered and applied in 4 injections to a reversed phase Zorbax preparative column. A 0 → 25% methanol in 0.1 M ammonium acetate buffer, pH 7.0 gradient for 1 hour with 8 ml/min flow rate was used. Among a total of 35 peaks collected, a fraction with a retention time of 50 minutes was pooled from the 4 injections. This was concentrated to 2.0 ml and purified on an Ultrasphere ODS C_{18} reversed phase column. The conditions used for de-

salting and purification were water for 30 min followed by a 0 → 25% water-methanol gradient for 1 hour with a flow rate of 4 ml/min. The purified peaks (3 A_{249}) with a retention time of 40 minutes had a UV spectra identical to those of nucleoside I. An NMR spectrum and co-injections of this material with nucleoside I and authentic 5'-deoxyinosine by HPLC in the 4 solvent systems A, B, C and D confirmed the identity as 5'-deoxyinosine.

Preparation of 5'-deoxyinosine. 5'-Deoxyadenosine was deaminated to 5'-deoxyinosine by a modification of the method of Shapiro and Pohl[14]. Ten mg of 5'-deoxyadenosine dissolved in 4 ml 3.5 N acetic acid was treated with 4 ml of 4 N $NaNO_2$, and allowed to stand at 25°C for 3 hours. The reaction mixture was concentrated, adjusted to pH 4 and fractionated by HPLC. The desired product, 5'-deoxyinosine was eluted at 30 minutes when chromatographed on a reversed phase Ultrasphere ODS C_{18} preparative column using a 0 → 25% water-methanol gradient in 70 minute with a 4 ml/min flow rate. The starting material, 5'-deoxyadenosine eluted at the end of the gradient. This material was identical to the authentic sample when compared by UV and NMR spectra and eluted as a single peak in HPLC when coinjected with the authentic sample.

Preparation of 3'-deoxyinosine and 2'-O-methylinosine. To five mg of 3'-deoxyadenosine dissolved in 5 ml of potassium phosphate buffer (pH 7.6) was added 0.1 ml (2 mg/ml in phosphate buffer) (36 units) of adenosine deaminase. The rate of the reaction was monitored by TLC on silica gel G plates in solvents a and b, and also by noting the change of OD at 265 nm. After 18 hours at 22°C, the reaction mixture was lyophilized and the products dissolved in 2 ml of water and purified by a reversed phase Magnum 9 ODS-2 C_{18} column using a 2 →30% water-methanol gradient in 30 min with a 3 ml/min flow rate. The desired product eluted after 25.0 min and the starting material was removed with a 50% water-methanol wash.

Using the above procedure 2'-O-methylinosine was prepared by deamination of 5 mg of 2'-O-methyladenosine. The 2'-O-methylinosine was purified by using a 0 → 40% water-methanol gradient (1 hour), with a flow rate of 4 ml/min, on an Ultrasphere ODS C_{18} reversed phase preparative column. The 2'-O-methylinosine (3.6 mg) eluted after 20 min while the starting material eluted at 50 min. The UV and NMR spectra of the products established the structures of these materials.

Acid hydrolysis of nucleoside I. A solution of 5.0 A_{248} units of nucleoside I in 250 µl of 0.1 N HCl was heated at 100° for 30 min. The reaction mixture when analyzed by UV and paper chromatography showed that hypoxanthine (3.5 A_{260}) was formed as the main product.

RESULTS

Characterization. In UV spectra the unknown urinary nucleoside I, exhibited maxima at 249 nm at pH 5.9 and 1.0 while at pH 11.0 the maxima shifted to 253 nm (FIG. 2a). These UV spectral maxima and other subtle characteristics were similar to those found in the UV spectra of inosine, 2'-deoxyinosine, 2'-O-methylinosine, 3'-deoxyinosine and the spectra were most similar to those of the 5'-deoxyinosine (FIG. 2b). From the results it was obvious that the compound was closely related to inosine.

Nucleoside I was converted to the volatile trimethylsilyl (TMS) derivative, and both low and high resolution mass spectra were obtained. The assignments shown in FIG. 3a and TABLE 1 are based on exact mass values of the principal diagnostic ions and on earlier studies of the spectra of model nucleosides[15]. The number of TMS groups introduced was 3, established by a separate experiment using [$^2H_9$]TMS blocking groups[16]. All assignments shown are supported by appropriate mass shifts from the spectrum of the deuterated derivative.

The M-CH_3 ion composition of $C_{18}H_{33}N_4O_4Si_3$ corresponds to the tris(TMS) derivative of nucleoside I and shows the composition of underivatized nucleoside I to be $C_{10}H_{12}N_4O_4$. The base series of ions[15] of m/z 193, 208, 209, 237, 281, 323 and 335 show a composition corresponding to the base hypoxanthine, while the characteristic sugar ions at m/z 260, 245, 232 and 171 (S-TMSOH) unambiguously reveal the sugar to be a deoxypentose. The B+116 ion specifically includes O-2' from the sugar, thus excluding 2'-deoxyinosine as the structure of nucleoside I. This conclusion is supported by significant differences in the mass spectra of authentic 2'-deoxyinosine-$(TMS)_3$, and by gas chromatographic retention times: 2 min., 3 sec. for I-$(TMS)_3$ vs. 3 min. 58 sec. for 2'-deoxyinosine-$(TMS)_3$. That nucleoside I is 5'-deoxyinosine or its alpha-anomer is suggested by the low abundance in FIG. 3a of m/z 103, which in normal nucleosides is due to 5'-CH_2OTMS. The mass spectrum from I was therefore compared with that of authentic

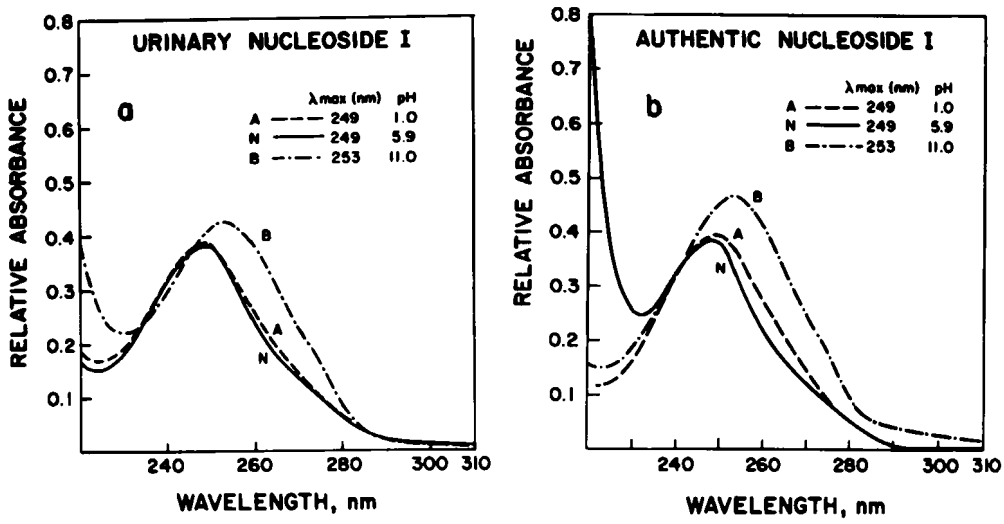

FIG. 2. UV spectra of (a) urinary nucleoside I and (b) authentic 5'-deoxyinosine.

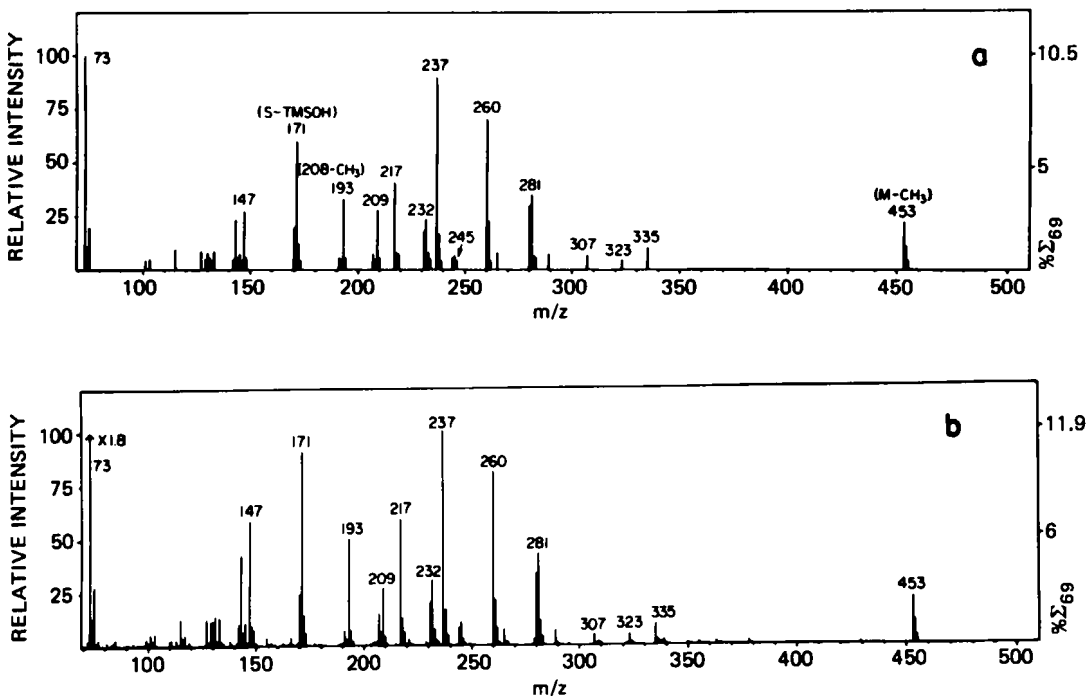

FIG. 3. Mass spectrum of trimethylsilyl-derivative of (a) urinary nucleoside I, (b) authentic 5'-deoxyinosine $(TMS)_3$.

TABLE 1

Structure Assignments from the High Resolution Mass Spectrum of Trimethylsilylated Urinary Nucleoside I

Observed Mass	Error, mmu*	Elemental Composition	Assignment†
453.1834	2.4	$C_{18}H_{33}N_4O_4Si_3$	M-CH_3
323.1347	-1.2	$C_{16}H_{33}N_4O_2Si_2$	B+116
281.1236	-1.8	$C_{11}H_{21}N_4OSi_2$	B+74
260.1255	-0.9	$C_{11}H_{24}O_3Si_2$	S-H
245.1036	0.7	$C_{10}H_{21}O_3Si_2$	S-H-CH_3
237.0820	1.2	$C_9H_{13}N_4O_2Si$	B+30
232.1304	-1.1	$C_{10}H_{24}O_2Si_2$	S-H-CH_2O
209.0867	0.8	$C_8H_{13}N_4OSi$	B+2H
208.0784	0.4	$C_8H_{12}N_4OSi$	B+H

*Difference in millimass units, observed minus theoretical values.
†M, molecular ion; B, base fragment; S, sugar fragment. Further details regarding assignments appear in ref. (15).

5'-deoxyinosine-$(TMS)_3$ (FIG. 3b). The spectra are experimentally indistinguishable, thus supporting the identity of I as 5'-deoxyinosine. These data also exclude the alpha-anomer, which is expected to exhibit different ion abundances, based on comparison of other alpha-beta anomeric pairs[15,17].

The NMR spectrum of the unknown nucleoside I exhibited a doublet for CH_3 group at 1.45 ppm. In addition, C_2 and C_8 protons were observed at 8.20 and 8.22 ppm respectively, further supporting the hypoxanthine moiety in the base portion of the molecule. A doublet for the anomeric proton was observed at 6.04 ppm. On the whole the proton magnetic resonance spectra of urinary unknown nucleoside I were similar to those of the 5'-deoxyinosine (TABLE 2). The resonance observed at 1.45 ppm for a CH_3 group differentiated it from inosine, 2'-deoxyinosine, 3'-deoxyinosine and 2'-O-methylinosine.

The naturally occurring nucleoside I and authentic 5'-deoxyinosine were compared by TLC and HPLC. In TLC, the two samples were identical in five solvents (TABLE 3). In HPLC, the unknown urinary nucleoside I had a retention time different from those of inosine, 2'-deoxyinosine, 3'-deoxyinosine and 2'-O-methylinosine in solvent B. The material, however, behaved very similar to 5'-deoxyinosine which was injected

TABLE 2
Chemical Shifts for Unknown Urinary Nucleoside I and 5'-Deoxyinosine in D_2O

	C_8-H	C_2-H	C_1'-H	C_3'-H,C_4'-H	C_5'-CH_3
Unknown Urinary Nucleoside I	8.22(s)	8.20(s)	6.04(d)	4.20(m)	1.45(d)
5'-Deoxyinosine	8.26(s)	8.23(s)	6.05(d)	4.20(m)	1.45(d)

TABLE 3

TLC Comparison of the Unknown Urinary Nucleoside I and II with Authentic 5'-Deoxyinosine

	Rf x 100 Solvent Systems*				
	a	b	c	d	g
Unknown urinary nucleoside I	35	48	54	14	53
5'-Deoxyinosine	35	48	54	14	53

*Solvents are described in materials and methods section.

TABLE 4

HPLC Retention Time (Minutes) for Unknown Urinary Nucleoside I and 5'-Deoxyinosine

Compound	Solvent System* (A)	(B)	(C)	(D)
Unknown urinary nucleoside I	31.05	17.86	25.18	26.47
5'-Deoxyinosine	30.56	17.75	25.07	26.50
Mixture Coinjection (single peak)	31.07	18.00	25.62	26.85

*Solvents are described in materials and methods section.

individually as well as coinjected with the natural material I. In four solvents the coinjected mixtures eluted as homogenous single peaks identifying the unknown urinary nucleoside I as 5'-deoxyinosine. The retention times for the individual nucleosides as well as for the co-injections are listed in TABLE 4.

Urinary nucleoside II was isolated from the urine of a CML patient (R.W.) by paper chromatography in a manner similar to that described for nucleoside I. High resolution fast atom bombardment mass spectra of the

nucleoside II showed a protonated molecule of mass 314.0920 (MH^+, rel. int. 99%) corresponding to a molecular weight of 313.0842, or molecular composition $C_{11}H_{15}N_5O_4S$ (calc. 313.0844). The fragment ion m/z 136 (rel. int. 100%) corresponds to an unsubstituted adenine moiety[18]. Its identification as 5'-deoxy-5'-methylthioadenosine sulfoxide was confirmed by UV, GC/MS and NMR spectra comparisons with an authentic sample. The authentic sample was prepared from 5'-deoxy-5'-methylthioadenosine by a modification of the procedure of Schlenk et al.[19]. Identical retention times of both the natural and the authentic sample individually and by coinjection on HPLC in 4 solvent systems unequivocally confirmed the identity of nucleoside II as 5'-deoxy-5'-methylthioadenosine sulfoxide.

DISCUSSION

The new urinary nucleoside 5'-deoxyinosine (I) has been unequivocally characterized from the urines of two CML patients (2.1, 0.6 mg/24 hr urines). As discussed in the results section, structure of nucleoside I was assigned on the basis of UV, GC/MS, HPLC, TLC and NMR comparison with the authentic samples. It is the first 5'-deoxy nucleoside to be found in human urine. In terms of origin, 5'-deoxyinosine poses a real challenge since this nucleoside by itself is not known to occur in any biological systems. It cannot be a part of nucleic acids since the 5'-hydroxyl group essential for the 3',5'-phosphodiester linkage of the nucleic acids is absent in this molecule.

One possible source of 5'-deoxyinosine in mammals appears to be 5'-deoxyadenosine which is liberated from coenzyme-vitamin B_{12} through enzyme induced homolytic cleavage of the labile cobalt-carbon bond[20]. This cleavage leads to the formation of 5'-deoxyadenosyl radical $AdCH_2^{\cdot}$ followed by the abstraction of a hydrogen atom from the substrate to generate substrate radical and 5'-deoxyadenosine. It may seem that the deamination of 5'-deoxyadenosine could give rise to 5'-deoxyinosine, however, the former is a very poor substrate of adenosine deaminase[21] and thus very unlikely to deaminate in mammalian systems. Recently it has been recognized that 5'-deoxyadenosine which was thought to be an inert nucleoside is an excellent substrate for the enzyme 5'-deoxy-5'-methylthioadenosine phosphorylase[22]. The latter enzyme[23] has attracted a great deal of attention since it cleaves in addition to

5'-deoxy-5'-methylthioadenosine (MTA) a number of other adenosine derivatives and produces a modified adenine and modified ribose-1-phosphate depending upon the analogs[24,25]. The 5'-deoxyadenosine is cleaved by MTA phosphorylase to give adenine and 5'-deoxyribose-1-phosphate. The latter in the presence of hypoxanthine and purine nucleoside phosphorylase could give rise to 5'-deoxyinosine as found in cultured cells[26].

Mills et al. have suggested that 5'-deoxy-5'-methylthioadenosine sulfoxide, nucleoside II, may arise from MTA by *in vivo* oxidation with peroxides and superoxides or enzymatically by liver microsomes[12]. Backlund[27] et al. have reported that MTA is converted to methionine by rat liver homogenate in the presence of magnesium, however in the absence of magnesium an unknown compound suggested to be the nucleoside II accumulated in this system[12]. It is well established that vitamin B_{12} is also involved in the maintenance of methionine levels by transmethylation of homocysteine to methionine through a vitamin B_{12} mediated enzyme, methionine synthetase. Methionine is converted to S-adenosylmethionine and the latter releases 5'-deoxy-5'-methylthioadenosine (MTA) during polyamine biosynthesis[28]. MTA in turn can be recycled to methionine or as suggested above can be converted to 5'-deoxy-5'-methylthioadenosine sulfoxide.

The liberation of 5'-deoxyadenosine, a precursor of nucleoside I, has been associated with the only other known vitamin B_{12} dependent mammalian mutase enzyme, i.e. the conversion of methylmalonyl CoA to succinic acid[20,29]. Recently, we have identified 7-β-D-ribofuranosylhypoxanthine in the urine of a CML patient and postulated an analogue of vitamin B_{12} as a possible source of this nucleoside[7]. Thus, the finding of these three nucleosides associated with vitamin B_{12} and its two mammalian regulatory enzyme systems may be related to the abnormally high levels of vitamin B_{12} observed in CML serum[30,31]. A correlation may exist between excreted levels of these urinary nucleosides and levels of vitamin B_{12} and MTA phosphorylase enzyme[31] in CML as well as other myeloproliferative diseases.

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