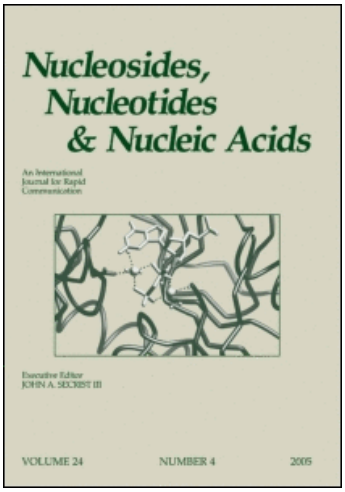

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Isolation and Identification of 1-Ribosyl Pyridone Nucleosides from Human Urine

Gordon C. Mills[a]; Nora J. Davis[a]; Khingkan Lertratanangkoon[a]
[a] Division of Biochemistry, Department of Human Biological Chemistry and Genetics, and the Department of Pharmacology, University of Texas Medical Branch, Galveston, Texas

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ISOLATION AND IDENTIFICATION OF 1-RIBOSYL PYRIDONE NUCLEOSIDES FROM HUMAN URINE

Gordon C. Mills*, Nora J. Davis and Khingkan Lertratanangkoon
Division of Biochemistry, Department of Human Biological Chemistry and Genetics, and the Department of Pharmacology, University of Texas Medical Branch, Galveston, Texas 77550

Abstract - 1-Ribosylpyridin-4-one-3-carboxamide with lesser amounts of 1-ribosylpyridin-2-one-5-carboxamide have been isolated quantitatively from human urine using ion exchange column chromatography. Absorption spectra and mass spectrometry were used in the identification.

INTRODUCTION

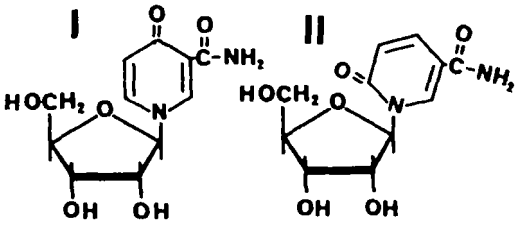

FIG. 1. I, Ribosylpyridin-4-one-3-carboxamide. II, 1-Ribosylpyridin-2-one-5-carboxamide.

In previous studies from this laboratory, we have utilized ion exchange chromatography to purify and identify a number of bases and nucleosides in human urine[1]. An XAD-4 desalting column[2,3] has facilitated rechromatography of many of these compounds during the purification procedures. One compound that had been previously noted in many of the urine specimens, has now been identified as 1-ribosylpyridin-4-one-3-carboxamide (4,3-Py-R). This compound is identical to that isolated and characterized by Dutta and coworkers[4] using an entirely different separative technique. The previous authors obtained low yields of the compound from ca. three liters of urine. In our procedures, we utilized ca. 10 - 20 ml of urine with quantitative recoveries. We have also identified and quantitatively determined small amounts of an isomer, 1-ribosylpyridin-2-one-5-carboxamide (2,5-Py-R), in urine.

A urinary ribosyl pyridone has also been reported by Gehrke, Kuo and coworkers[5,6], and by Mrochek, et al[7]. Both groups tentatively identified the compound as 1-ribosylpyridin-2-one-5-carboxamide. After exchanging samples and data with Drs. Gehrke and Kuo, it is now evident that the ribosyl pyridone isolated by these workers (designated GN or PCNR) is also 4,3-Py-R instead of 2,5-Py-R. We presume that the compound noted by Mrochek, et al[7] was also 4,3-Py-R, since their experiments would not have differentiated between the two isomers.

MATERIALS AND METHODS

Chemicals and Solutions

Analytic grade ion exchange resins (AG1-X4 and AG50-X4, 200/400 mesh) were obtained from Bio-Rad Laboratories, XAD-4 (20/50 mesh) from Mallinckrodt Chemical Works, nicotinamide mononucleotide (NMN) and bovine intestinal alkaline phosphatase, Type VII-NA, from Sigma Chemical Co. and N,O-bis(trimethylsilyl)acetamide from Pierce Chemical Co. All components of buffers were reagent grade, and pH values are at 23°C. The listed concentration of the sodium acetate buffer refers to the sodium concentration. The sodium borate solution (0.010 M) was prepared from $Na_2B_4O_7 \cdot 10\ H_2O$ (3.81 g/liter). The NH_4Cl-NH_4OH-borate buffers were prepared by adjustment of the NH_4Cl-borate solution to pH 10.6 with NH_4OH.

Analytical Methods

The orcinol reaction for ribose was carried out with heating for 20 min at 100°C using the orcinol-$FeCl_3$-HCl reagent prepared as described by Brown[8]. Total phosphate determination in synthetic pyridone nucleotides was carried out after sulfuric acid digestion as described previously[9]. Millimolar absorbancy values for synthetic pyridone nucleotides were calculated from ultraviolet absorbance values, using total phosphate assays to determine nucleotide concentrations. The pyridone nucleotides were converted to the corresponding nucleosides with no change in absorbance values. Products in elution profiles were routinely identified by absorbance measurements at 240, 250, 260, 275 and 290 nm, with additional readings (e.g., 320 nm) where appropriate. A Gilford spectrophotometer (Model 222) was used for spectrophotometric measurements, while an Aminco-Bowman spectrophotofluorometer was used for fluorescence measurements.

Procedures for Column Chromatography of Pyridone Nucleosides.

Procedure A. The sample, with a very low salt content and a volume of 6 - 8 ml, was applied to an AG50-X4 column, H^+ form, 0.50 cm x 40 cm. Elution was carried out using a 0.077 M, pH 4.8 sodium acetate buffer. Fraction volumes were 3 - 4 ml with a flow rate of ca. 0.20 ml/min.

Procedure B. The AG1-X4 column (0.50 cm x 40 cm) was equilibrated with a 0.125 M NH_4Cl + NH_4OH + 0.010 M borate buffer, pH 10.6 (Buffer A), followed by a 0.025 M NH_4Cl + NH_4OH + 0.010 M borate buffer, pH 10.6 (Buffer B). The desalted sample with a volume of 6-8 ml (including 1 ml of Buffer B), was adjusted to pH 10.4 - 10.6. After application of the sample to the column, gradient elution was carried out with 75 ml of Buffer B in the closed mixing reservoir and 150 ml of Buffer A in the upper reservoir. Fraction volumes were 3 - 3.5 ml with a flow rate of ca. 0.20 ml/min.

Procedure C. The sample (usually pooled fractions from another column) was adjusted to pH 6 - 7.5, concentrated to ca. 10 ml, and applied to the XAD-4 column (1.3 cm x 30 cm). Elution was carried out with water (ca. 25 ml), then with 19% (v/v) ethanol. Fraction volumes ranged from 10 to 5 ml with flow rates ranging from 1.0 to 0.50 ml/min.

Procedure D. The desalted sample was applied to the AG50-X4 column (0.50 cm x 40 cm, H^+ form) in 0.01 N HCl (volume, 6 - 8 ml). Gradient elution was carried out with 75 ml of 0.01 N HCl in the closed mixing reservoir and 100 ml of 1.5 N HCl in the upper reservoir. Fraction volumes were ca. 3.5 ml with a flow rate of ca. 0.20 ml/min.

Gas Chromatography (GC) and Mass Spectrometry (MS) Analyses.

Direct chemical ionization MS. A small amount of the underivatized sample was analyzed by direct chemical ionization (CI) in the positive mode with methane as reagent gas. The mass spectrometer employed was a Nermag R 10-10 C equipped with a PDP 11/73 data system. The ion source temperature was 100°C and the ionizing voltage was 70 ev. The CI filament was temperature programmed from 0 mA to 500 mA at 20 mA/sec. The sample was introduced by direct probe.

Preparation of trimethylsilylated derivative. An aliquot of the dried sample was dissolved in pyridine and trimethylsilylated with bis(trimethylsilyl)acetamide. The reaction was carried out at 60°C for 1 hr and an aliquot was analyzed by GC/MS.

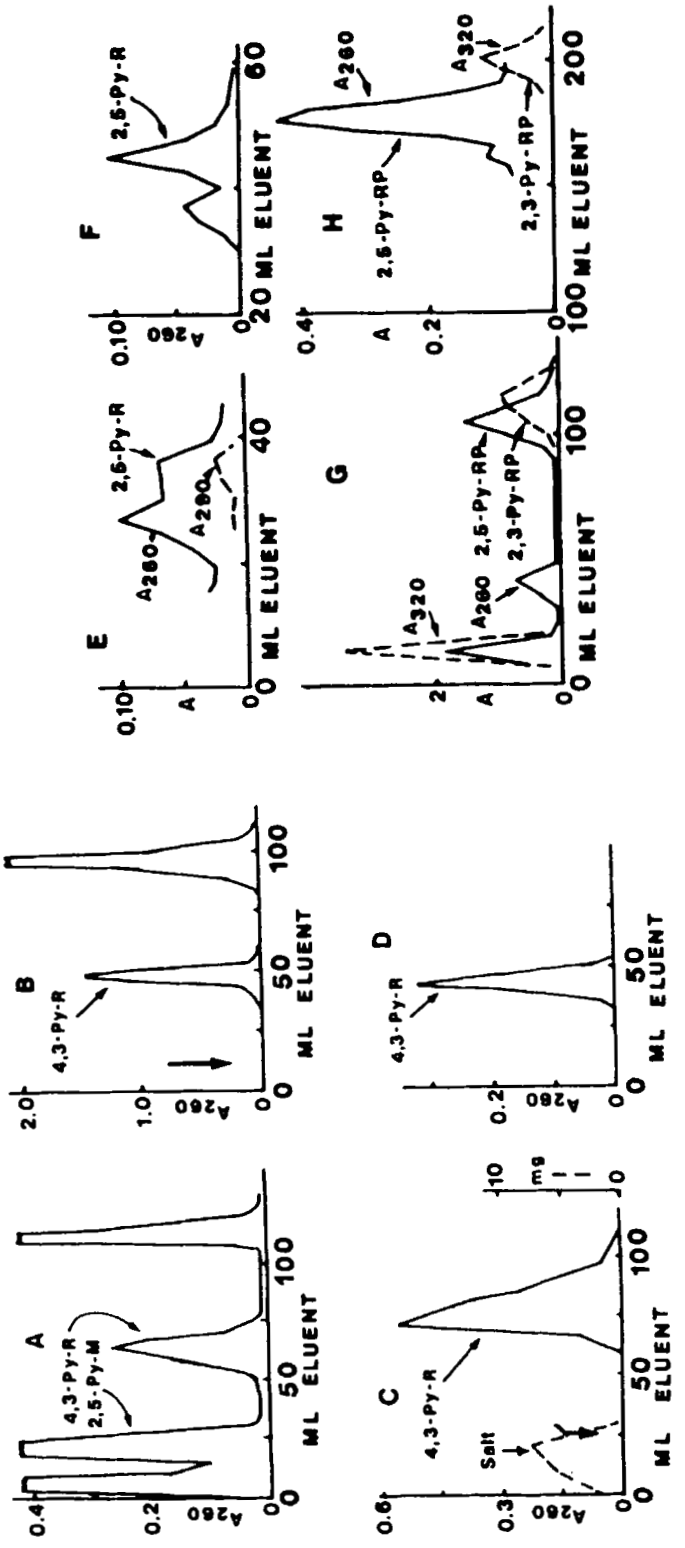

FIG. 2. Illustrations of column separations.

A. Buffer elution of Fraction CW using an AG50-X4 cation exchange column (Procedure A). The eluant pH rose sharply from pH 2.8 to 4.8 at 108 ml of eluent, and a major portion of the purines and pyrimidines were eluted at that point.

B. Separation of 4,3-Py-R and 3,7-dimethylxanthine by elution from an AG1-X4 anion exchange column at pH 10.6 in the presence of borate (Procedure B). In the absence of borate, 4,3-Py-R was not retained and was eluted as shown by the arrow.

C. Separation of 4,3-Py-R from salt using an XAD-4 resin (Procedure C). The column was eluted first with water, then with 19% ethanol (v/v) as indicated by the arrow. Salt is expressed as mg/0.3 ml.

D. Elution of 4,3-Py-R from an AG50-X4 cation exchange column using an HCl gradient (0.01 M → 1.5 M) (Procedure D).

E. Separation of isolated 2,5-Py-R from other compounds on an AG1-X4 anion exchange column at pH 10.6 in the presence of borate. Procedure B was modified to give a steeper NH_4Cl gradient (0.025 M → 0.25 M).

F. Rechromatography of pooled isolated 2,5-Py-R samples (Procedure B).

G. Separation of the oxidation products of NMN on a 0.5 x 20 cm AG1-X4 anion exchange column using formic acid elution (for details, see text).

H. Purification of synthetic 2,5-Py-RP by rechromatography on a 0.5 x 40 cm AG1-X4 anion exchange column using formic acid elution (for details, see the text).

GC/MS analysis. GC/MS analysis was carried out with a Nermag R 10-10 C/PDP 11-73 equipped with a Varian 3400 gas chromatograph. The GC column was a DB-5 (15 m x 0.25 mm i.d.) fused silica capillary column (J & W Scientific, Inc.). Helium was used as carrier gas and the separation was temperature programmed from 100°C to 280°C at either 10°C/min or 20°C/min. The ion source temperature was 200°C and the ionizing energy was 70 eV. The mass spectrometer was operated in the positive chemical ionization mode with methane as reagent gas.

RESULTS

Isolation of 4,3-Py-R and 2,5-Py-R from Urine.

Fraction CW from urine, which contained compounds with no charge at pH 10, was obtained as described previously[1]. Fraction CW was applied to a cation exchange column, H^+ form, and eluted with a pH 4.8 sodium acetate buffer (Procedure A, Fig. 2A). The 2,5-Py-R is not retained on the column and comes through in the column wash and in the first 15 ml of eluate. The major pyridone in urine, 1-methylpyridin-2-one-5-carboxamide (2,5-Py-M) is retained very slightly (probably by adsorption) and is eluted after 2,5-Py-R. The 4,3-Py-R peak in this profile was occasionally contaminated with theobromine (3,7-dimethylxanthine). The effluent (dilute acetic acid) was removed by evaporation from pooled fractions containing 2,5-Py-R and from pooled, 4,3-Py-R fractions. The 4,3-Py-R fraction and the 2,5-Py-R fraction were then rechromatographed separately on an AG1-X4 anion exchange column operated at pH 10.6 in the presence of 0.010 M borate (Procedure B, Fig. 2B and Fig. 2E). Since borate complexes with the ribose moiety to give a minus one charge, the ribosyl pyridones are retained on the column and are eluted with the pH 10.6 buffer-borate solution. After desalting with an XAD-4 column (Procedure C, Fig. 2C), fractions containing 4,3-Py-R were applied to an AG50-X4 cation exchange column and eluted with HCl (Procedure D, Fig. 2D). The fractions containing 2,5-Py-R were desalted with a XAD-4 column and rechromatographed on the AG1-X4 column with pH 10.6 buffer-borate elution (Procedure B, Fig. 2F). After desalting, the purified 4,3-Py-R and the 2,5-Py-R were used for obtaining ultraviolet absorption spectra, for mass spectrometry studies and for other chemical studies.

Synthesis of 2,5-Py-R from NMN.

NMN was oxidized to a mixture of pyridones using alkaline ferricyanide. The conditions utilized were those described by Pullman and Colowick[10] for preparation of 1-methylpyridones from 1-methylnicotinamide. The reaction was carried out at room temperature with stirring using 40 μmols of potassium ferricyanide and 20 μmols of NMN in 10 ml 0.50 N NaOH. The reaction was stopped after 3 min by adjusting the pH to ca. 7 with HCl, prior to application to an anion exchange column (AG1-X4, 0.50 x 20 cm, formate form). Elution was carried out with fraction collection (Fig. 2G) using a formic acid gradient (0 → 4.0 N; 75 ml water initially in a closed mixing reservoir). The alkaline conditions in the oxidizing reaction mixture caused considerable cleavage of the nicotinamide-ribose bond, so the mixed pyridone nucleotides of NMN were obtained in only ca. 40 per cent yield. Ultraviolet absorbing cleavage products no longer contained the phosphate moiety, so they were eluted early from the anion exchange column (0-40 ml) while the desired pyridone nucleotides were eluted later (90-125 ml). Fractions containing the desired pyridone were pooled and formic acid was removed by evaporation. The compounds were dissolved and applied to a second anion exchange column (0.50 cm x 40 cm, AG1-X4, formate form) and again eluted using a formic acid gradient (Fig. 2H). Water (75 ml) was initially in the closed mixing reservoir with 4 N formic acid in the upper reservoir. This separation provided pure samples of 1-(5'-phosphoribosyl)-pyridin-2-one-5-carboxamide (2,5-Py-RP) and 1-(5'-phosphoribosyl)-pyridin-2-one-3-carboxamide (2,3-Py-RP) after removal of formic acid by evaporation. No 4,3-Py-RP was found in the elution profiles as a product of the alkaline ferricyanide reaction. The 2,5-Py-RP and 2,3-Py-RP could be readily identified by the marked differences in their absorption spectra (Fig. 3) and by the fact that only 2,3-Py-RP fluoresces at neutral pH (excitation, 330 nm; fluorescence, 385 nm). Both compounds also contained ribose and phosphate as shown by appropriate assays (see Methods).

Each of the synthetic pyridone nucleotides (2,5-Py-RP and 2,3-Py-RP) was converted to the corresponding nucleoside by incubation with alkaline phosphatase (0.9 unit/ml sample) for 6 hr at 37 °C in pH 9.4, 0.05 M Tris buffer, with 2 mM Mg^{++}. The nucleoside was recovered in each case in ca. 100% yield following desalting on an XAD-4

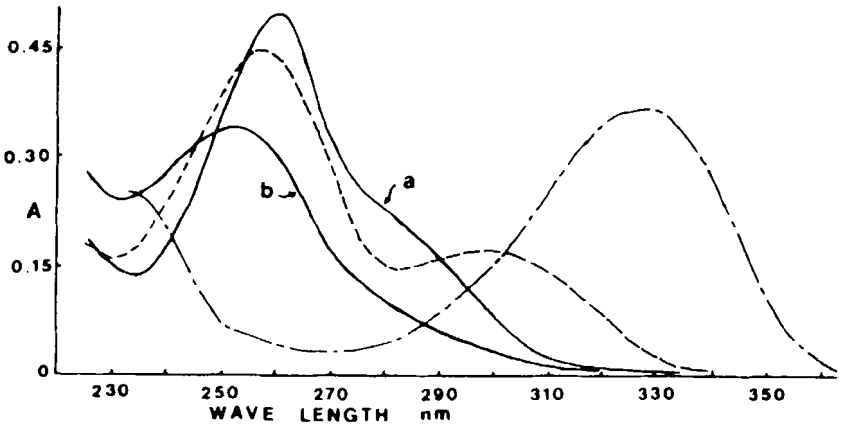

FIG. 3. Ultraviolet absorption spectra of the three isomeric pyridone nucleosides.

Solid lines, 4,3-Py-R (0.0315 μmol/ml) a, at neutral pH; b, in 1 N HCl. Dashed-line, 2,5-Py-R at neutral pH (0.032 μmol/ml). Dot-dashed line, 2,3-Py-R at neutral pH (0.034 μmol/ml). The nucleotides of 2,5-Py-R and 2,3-Py-R have nearly identical spectra to that shown for the corresponding nucleosides.

column. Each nucleoside was rechromatographed on an AG1-X4 column (pH 10.6, with borate, Procedure B). After removal of ammonia by evaporation, the samples were again desalted prior to using the 2,5-Py-R and 2,3-Py-R for spectroscopic (Fig. 3, Table 2), chemical (Table 1), or mass spectrometry studies.

The synthesis of 2,5-Py-R has been described recently by Frister, et al[11] starting with pyridin-2-one-5-carboxamide. These workers characterized their synthetic compound by EI mass spectra, NMR spectra and ultraviolet absorption spectra. Our synthetic 2,5-Py-R has been shown by Drs. Gehrke and Kuo to be identical in regard to ultraviolet absorption spectra and HPLC retention times with the synthetic 2,5-Py-R provided by Dr. Schlimme.

TABLE 1. Comparison of Properties of Ribosyl Pyridones

	Orcinol reaction for ribose#	AG1 pH 10.6 without borate	AG1 pH 10.6 with borate	Fluorescence	Shift of maximum absorbance in HCl
Isolated 4,3-Py-R	9	Not Retained	Retained	None	260 nm to 252 nm
Authentic 2,5-Py-R	117	Not Retained	Retained	None	None
Isolated 2,5-Py-R	106	Not Retained	Retained	None	None
Authentic 2,3-Py-R	127	Not Retained	Retained	385 nm (330 nm excit.)	None

#Expressed as per cent of color yield of adenosine with 20 min heating at 100°C.

TABLE 2. Comparison of Spectral Properties of Methyl and Ribosyl Pyridones

Compound	pH	Max nm	A^{Max}_{mM} [a]	$\frac{240}{260}$	$\frac{275}{260}$	$\frac{290}{260}$	$\frac{320}{260}$
4,3-Py-R	7	260	16[b]	0.34	0.52	0.33	0.00
"	0	252	10.8	0.92	0.43	0.21	0.00
4,3-Py-M	7	256	13.2[c]	0.30	0.43	0.40	0.02
"	0	240	9.0[c]	2.00	0.46	0.22	0.07
2,5-Py-R	7	258	14.3	0.52	0.42	0.36	0.18
2,5-Py-M	7	260	14.2[d]	0.44	0.45	0.34	0.09
2,3-Py-R	7	327	10.7	4.8	0.9	2.0	8.1
2,3-Py-M[d]	7	322	8.9	--	1.1	2.9	12

[a]Millimolar absorbancy at the maximal wavelength. [b]Value from Ref. 14.

[c]Value from Ref. 14. [d]Value from Ref. 10.

Other Properties of Isolated and Synthetic Pyridone Nucleosides.

Our isolated 4,3-Py-R gave only a minimal reaction for ribose by the orcinol procedure (0.09 μmol ribose/μmol compound (Table 1). Dutta, et al[4] reported that 4,3-Py-R gave a positive orcinol test for ribose. However, they provided no quantitative data with appropriate standards, so their report does not necessarily conflict with our data. In contrast, ribose moieties of 2,5-Py-R and 2,3-Py-R react readily in the orcinol procedure (Table I). Studies on acid lability of the nitrogen-ribose bond of isolated 4,3-Py-R indicated that the bond was much more stable to acid than the purine-ribose link of nucleosides[12]. About 40 per cent of the starting compound was recovered unchanged after heating 1 hour at 100° C in 1 N HCl. This stability to acid hydrolysis may at least partially explain the low yield of ribose from 4,3-Py-R in the orcinol assay. The spectra of 4,3-Py-R (Fig. 3) is similar to that noted previously by Dutta, et al[4], except for the spectra in acid. Their acid spectra was carried out at pH 1.0, whereas our studies were in 1 N HCl. It is clear that 1 N HCl is required to produce the fully cationic form of 4,3-Py-R and hence their acid spectra (Fig. 1 of Ref. 4) is that of a mixture of cationic and neutral forms.

GC and GC/MS Studies on Isolated and Synthetic Pyridone Nucleosides.

The CI mass spectra of underivatized 4,3-Py-R and 2,5-Py-R are shown in Fig. 4 and Fig. 6A, respectively. The ion at m/z 271 corresponds to the protonated M + 1 molecular ion in each case, while the ion at m/z 239 for 4,3-Py-R results from the elimination of 32 amu or CH_3OH (presumably the C_5 of the ribose moiety) from the protonated molecular ion. Fragments indicative of the pyridone base structure (b) for each nucleoside are formed in analogy to fragmentation pathways described by McCloskey[13] for nucleosides: m/z 138, b + H; m/z 139, b + 2H; m/z 167, b + 30. Ions indicative of the sugar (S) moiety were also formed: m/z 133, S^+; m/z 115, $(S - H_2O)^+$. Other ions specific for the 4,3-Py-R are formed by the elimination of 17 amu (likely representing loss of NH_3) from m/z 167 and m/z 139 to form m/z 150 and m/z 122, respectively. These ions are barely discernible for the 2,5-Py-R CI spectrum.

Trimethylsilyl derivatives of 4,3-Py-R were prepared and the tris(trimethylsilyl) derivative was separated from the tetra(tri-

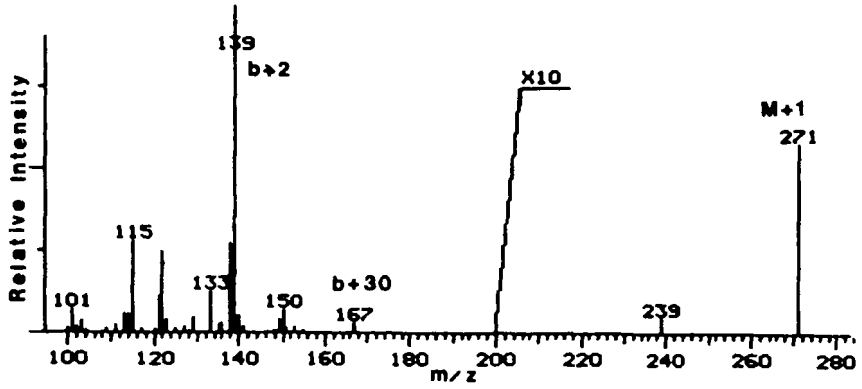

FIG. 4. CI mass spectrum of isolated 4,3-Py-R, with methane as reagent gas.

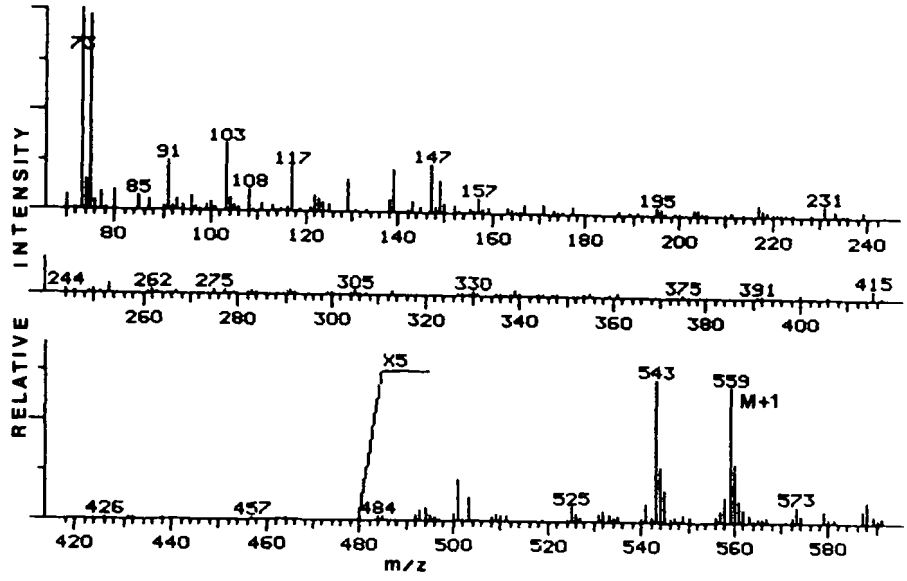

FIG. 5. CI mass spectrum of the tetra(trimethylsilyl) derivative of isolated 4,3-Py-R, with methane as reagent gas.

methylsilyl) derivative using capillary gas chromatography (see Methods). The CI mass spectra of the tris derivative of 4,3-Py-R (data not shown) clearly showed the protonated molecular ion at m/z 487. Fig. 5 shows the CI (methane) mass spectrum of the tetra(trimethylsilyl) derivative of 4,3-Py-R while the mass spectra of the corresponding derivative of 2,5-Py-R is shown in Fig. 6B. In each case, the intact molecule was observed as the protonated molecular ion at m/z 559. An ion at $(M + C_2H_5)^+$, m/z 587, was also observed. The protonated molecular ion indicates that the derivatives have four different trimethylsilyl groups (three on the ribose and the fourth on the amide moiety). The ion at m/z 543 in both cases corresponds to the loss of CH_4 from the protonated molecular ion.

The CI mass spectra of 2,5-Py-R and of trimethylsilylated 2,5-Py-R (Fig. 6) were similar to the corresponding spectra of 4,3-Py-R (Figs. 4 and 5) in some respects, particularly in regard to the larger ions. However, as expected for isomers, there were significant differences in relative amounts of many of the fragment ions. The CI mass spectrum of the isolated 2,5-Py-R (not shown) matched that of the synthetic compound in all of the major peaks, although the presence of small amounts of impurities were indicated. The trimethylsilylated derivative of the isolated 2,5-Py-R was also subjected to GC/MS. The CI mass spectrum of the tetra(trimethylsilyl) derivative of the isolated 2,5-Py-R (not shown) matched that of the synthetic compound perfectly.

Levels of Pyridone Nucleosides in Human Urine.

We have determined levels of 4,3-Py-R in fourteen random urine samples, five from males aged 14-25, with the others from small children. The excretion level (means $\pm$ S.D.) in the 14-25 yr group was 0.91 $\pm$ 0.16 nmol/μmol creatinine. In three normal children aged 4-6 yr, the 4,3-Py-R excretion ranged from 1.8 - 2.1 nmol/μmol creatinine, while in six children with immunodeficiency disorders, 4,3-Py-R excretion ranged from 1.5 - 7.7 nmol/μmol creatinine. The excretion level of 4,3-Py-R noted in our laboratory for adult males is very close to that reported previously for the same compound (designated GN) (0.87 $\pm$ 0.25 nmol/μmol creatinine) by Speer, Gehrke, et al[5].

The excretion level of 2,5-Py-R in three normal children was 0.16 - 0.19 nmol/μmol creatinine or about 7% of that of 4,3-Py-R. In

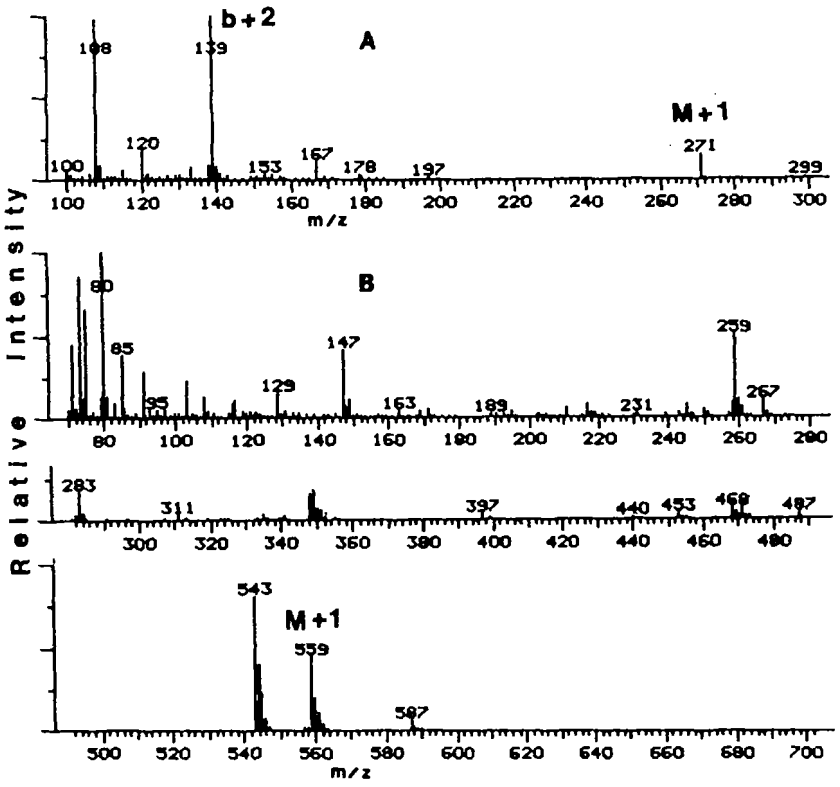

FIG. 6. A. CI mass spectrum of synthetic 2,5-Py-R, with methane as reagent gas. B. CI mass spectrum of the tetra(trimethylsilyl) derivative of 2,5-Py-R, with methane as reagent gas.

a pooled urine sample, the 2,5-Py-R level was 9% of that of 4,3-Py-R. A trace of 2,3-Py-R (ca. 1% of the 2,5-Py-R) was present in the isolated 2,5-Py-R as detected by its ultraviolet fluorescence. Thus, 4,3-Py-R makes up about 90% of the 1-ribosyl pyridones excreted in human urine, with 2,5-Py-R accounting for most of the remainder.

Recovery of Pyridones from Simulated Urine.

In order to check recoveries of 4,3-Py-R and 2,5-Py-R, we have added known amounts (0.25 - 0.30 μmols) of these two compounds and of 2,5-Py-M to 15 ml of simulated urine (urea plus salts). The 2,5-Py-M was included because it is the major urinary metabolite of dietary nicotinamide. The sample was then carried through the same separative procedures that were used for the urine samples. Percent

recoveries in two separate experiments were as follows: 4,3-Py-R, 91.2, 95.1; 2,5-Py-R, 83.0, 84.0; and 2,5-Py-M, 92.3, 96.2. These recoveries show that the amounts of the ribosyl pyridones isolated in our studies are indicative of the amounts actually present in the urine.

DISCUSSION

The fact that the isolated 4,3-Py-R is very weakly retained on a cation exchange column at pH 2.8 (Fig. 2A), but is retained much more strongly in the presence of HCl (Fig. 2D), indicates that 4,3-Py-R has a cationic pK_a of 2 or less. Similarly, the behavior on an anion exchange column of both the isolated ribosyl pyridones at pH 10.6 with and without borate (Fig. 2B and 2F, and Table 1) indicates that there is no anionic group in the absence of borate, but there are cis hydroxyl groups which will complex with borate to provide negatively charged complexes. The spectral characteristics of 4,3-Py-R in neutral solution and in HCl (Fig. 3 and Table 2) also indicate a pK_a of about 1 to 2. The lack of retention of 2,5-Py-R on a cation exchange column and identical spectra at pH 7 and in HCl, indicate that 2,5-Py-R will not form a cation.

The isomer assignment of the isolated 1-ribosyl pyridones is based primarily by analogy to properties of the three pyridone isomers produced from 1-methyl nicotinamide[14,15]. In that case, only 1-methyl-pyridin-4-one-3-carboxamide (4,3-Py-M) becomes cationic in an acid solution. Chang and Johnson [15] refer to this as a dipolar structure. When the keto group is on either carbon adjacent to the nitrogen of the pyridine ring, this dipolar cationic structure will not form. With both the 1-methyl and 1-ribosyl pyridones, the 4,3-isomer becomes cationic in acid solution with a corresponding shift of the absorption maxima to shorter wavelengths. This isomer does not fluoresce. The 2,5 isomer has an absorption maxima at about 260 nm, does not form a cation in acid, and does not fluoresce; while the 2,3-isomer has an absorption band at about 325 nm, does not form a cation, but it does fluoresce (Tables 1 and 2).

The results of MS and GC/MS studies are consistent with the conclusion that the major ribosyl pyridone that we have isolated from urine is 4,3-Py-R, and that the minor ribosyl pyridone is 2,5-Py-R. These studies provide correct molecular weights for the compounds (270 daltons);

they support the presence of an amide group; they indicate that there are three hydroxy groups and an amide group that may be trimethylsilylated; and they provide the correct mass number of the pyridone portion of the molecule. The linkage of the sugar to the pyridone has been previously established as the β-configuration for urinary 4,3-Py-R[4]. We presume there is also a similar β-linkage in the urinary 2,5-Py-R, since the authentic 2,5-Py-R used for comparison contained a β-linkage.

Pyridones of 1-methylnicotinamide have been known for many years to be major urinary metabolites of nicotinic acid and nicotinamide in man and most other mammals[16,17]. In man, 2,5-Py-M is the predominant urinary endproduct, being derived to some extent from the catabolism of NAD and NADP, but also more directly from surplus dietary nicotinamide or nicotinic acid. In contrast, urinary 4,3-Py-M in man appears to be derived almost entirely from the breakdown of NAD and NADP[14]. On a normal diet, 4,3-Py-M is excreted in humans at about one-third the level of its isomer, 2,5-Py-M. The third isomer, 1-methylpyridin-2-one-3-carboxamide (2,3-Py-M) is not excreted appreciably in man[18].

Based on the structures of the isolated 1-ribosyl pyridones, it seems reasonable to presume that they are catabolic products of one or both pyridine nucleotides, NAD and NADP. In the subjects we have studied, urinary excretion of 4,3-Py-R has been about one-half that of 4,3-Py-M. The relatively constant values for 4,3-Py-R excretion suggest that excretion levels are not affected by levels of dietary nicotinamide. Determination of urinary 4,3-Py-R might provide a useful measure of the rate of turnover of the pyridine nucleotide coenzymes NAD and NADP. The urinary levels found by Speer, et al[5] for the compound designated GN (4,3-Py-R) in patients with various types of cancer, suggests that excretion levels would indeed be of clinical value.

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