Urol Res (2005) 33: 231–238
DOI 10.1007/s00240-005-0467-5

ORIGINAL PAPER

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Uric acid monohydrate—a new urinary calculus phase

Received: 12 July 2004 / Accepted: 1 February 2005 / Published online: 29 May 2005

Abstract In our laboratory more than 100,000 urinary calculi have been analysed since 1972. Amongst this huge sample, 15 specimens originating from a total of eight patients were observed showing similar characteristics but escaping unambiguous identification with any of the substances that have been described so far in urinary concrements. Therefore, the unknown substance was submitted to a more extended analytical regimen. Structural analysis by x-ray crystallography turned out to be most successful, identifying the unknown material as uric acid monohydrate. Uric acid monohydrate crystallizes in the monocline space group $P2_1/c$. Within the crystal, uric acid and water molecules form continuous layers by hydrogen bonds. This is in contrast to uric acid in its water free and its dihydrate forms, which both crystallize by forming 3-dimensional networks To the best of our knowledge , the existence of a monohydrate form of uric acid has not been reported so far. Accordingly, this is the first report on uric acid monohydrate as a urinary stone component. The frequency of only 0.015% in our survey indicates that uric acid monohydrate is rarely the main component in concrements, in contrast to uric acid and uric acid dihydrate with frequencies of 10% and 6%, respectively. The infrared spectrum of uric acid monohydrate is very similar to that of the other crystal forms of uric acid. Because of this similarity and its low frequency, uric acid monohydrate may have been overlooked as a component of urinary concrements. X-ray diffraction allows for better differentiation in routine stone analysis. All samples of uric acid monohydrate were found by solid state NMR spectroscopy to be highly contaminated by amorphous material. This material consisted of long aliphatic chains reminiscent of lipids and fatty acids, respectively. Concrements consisting of other forms of uric acid or urate lacked this amorphous component. Therefore, a role of this aliphatic material has to be taken into consideration when discussing the conditions that may favour the rare formation of concrements from uric acid monohydrate. As for as the metabolic situation of the affected patients is concerned, no common peculiarities became evident by a retrospective survey.

Keywords Uric acid monohydrate · Urinary calculus · Crystal structure · Solid state NMR · X-ray diffraction · Infrared spectroscopy

Introduction

Usually urinary stones of alimentary or metabolic origin can be correctly identified by standard laboratory methods like infrared spectroscopy and x-ray diffraction. Respective stone components and their crystal forms are well known and comprehensive collections of their spectral and crystallographic data are available for the routine laboratory. Even complex mixtures of various stone components are thus accessible to correct analysis. Difficulties may arise rather from drug-induced concrements or from artefacts.

In our laboratory, using polarization microscopy, infrared spectroscopy and x-ray diffraction, more than 100,000 stones have been analysed[1]. However, there were a few samples that could not be identified unambiguously by spectral analysis. These samples, originating from different patients, had similar characteristics. Repeated analyses of stones from the same patient resulted in reproducible spectral data. By infrared spectroscopy, the respective samples showed similarities to the known concremental forms of uric acid. Therefore,

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an unknown crystal form of some metabolite of uric acid has been put forward as working hypothesis.

A more extended study on the true nature of the new substance became possible by cooperation with an institute experienced and equipped for highly specialised material analyses. By x-ray crystal structure analysis, the unknown substance was thus identified as uric acid monohydrate. This crystalline form of uric acid has been unknown to date. The respective crystallographic data are given in detail. For the routine laboratory, recommendations are given on how to differentiate uric acid monohydrate from other crystal forms of uric acid.

As far as the generation of uric acid monohydrate is concerned, the respective patients did not share any conspicuous clinical characteristics. On the other hand, there may be a crucial role of amorphous material consisting of long aliphatic chains. This material was found by solid state NMR spectroscopy only in concrements of uric acid monohydrate as the main component.

Materials and methods

Patients and urinary stones

A total of 15 stones was submitted for analysis between 1990 and 1999. The concrements originated from eight out patients (five males, three females) between 45 and 83 years of age. Three of the patients had experienced recurrent stone episodes (up to five) each resulting in apparently identical material. Twelve stones had passed spontaneously whereas three were removed instrumentally. On first detection 11 stones were localized in the kidney, two in the ureter and one each in the bladder and urethra. Clinical data from a retrospective request were scarce. Thus, five patients suffered from reduced kidney function by moderately elevated creatinine levels. Only the patient experiencing five stone episodes suffered from terminal renal failure.

Five patients had repeatedly acid urine pH values (≤ 5.5). Uric acid levels in serum and urine were, as far available, in the normal range.

Scanning electron microscopy

Stone morphology was studied by electron microscopy of gold coated samples of stone fragments on a scanning electron microscope SEM S360 (Cambridge Instruments) at 20 kV.

Infrared spectroscopy

Infrared spectra were recorded on a FTIR spectrometer Avatar 320 (Nicolet) at 4 cm^{-1} resolution and 32 scans per measurement. For sample preparation the KBr pellet technique was applied.

Elementary analysis

Elementary composition of stone material was studied by optical emission spectral analysis on a spectrograph Q24 (Carl Zeiss, Jena). Supportive data were derived from an energy dispersive x-ray (EDX) analysis employing an electron microprobe SEMQ (ARL).

X-ray diffraction and structure analysis

Routine x-ray diffraction of urinary stones was performed on a Seifert two-circle diffractometer XRD 3003 using Ni-filtered CuKα radiation. The measuring conditions for the determination of the lattice constants were as follows: 50 kV, 40 mA, angle range 4–100° 2Θ, step width 0.02°, count time 20 s. The evaluation of these data for structural analysis was based on several commercial programs: CRYSFIRE [2] and Powder Cell [3] for determination of the lattice constants and of the crystal symmetry, PATSEE [4] for determining the atomic coordinates, CAVITY [5] for screening for cavities within the modeled crystal structure and SHELX-97 [6] for refining the structure model of crystals composed of uric acid monohydrate.

Solid state NMR measurements

^{13}C-CP/MAS spectra of several samples of the unknown substance and of urinary stones of uric acid, uric acid dihydrate and acid ammonium urate were taken on a DMX 400 widebore spectrometer (Bruker) with an observation frequency of 100.13 MHz and a ^{1}H frequency of 400.63 MHz for CP and decoupling.

Hartmann-Hahn match and shift standardisation ($\delta^{\mathrm{TMS}} = 0$) were performed with a sample of glycine. Experimental parameters were 25 kHz sweep width, 1 ms contact time, Ramp-CP, a relaxation delay auf 3 s and acquisition time of 50 ms. The number of scans made was about 2,000. The data points were TD 2 K and SI 8 K, 5 Hz line broadening was applied.

Results

Stones morphology

Stone samples of unknown composition had the following morphological characteristics in common: individual samples consisted of small fragments weighing 0.05–0.5 g. They presented as porous concrements of light greyish-brown colour and of poor mechanical stability. Under the polarization microscope, grain preparations presented as uncharacteristic crystalline aggregates of strong birefringence. By contrast, the scanning electron microscopical examination resulted in a distinct structural pattern showing aggregates of plate-like crystals approximately 0.5–1.0 μm in height (Fig. 1a).

Infrared spectroscopy

The encounter with some unknown material became evident primarily from infrared spectroscopy as the main routine procedure in stone analysis. All 15 samples resulted in uniform spectra that differed significantly from the infrared spectra of all known components of urinary stones described so far. By screening relevant libraries (Aldrich, Merck, Sadtler) there was no match to be found to any of the listed spectra. However, there is some evident similarity of the infrared spectrum of the unknown substance to that of purine components like uric acid and uric acid dihydrate (Fig. 2) as well as xanthine and 2,8-dihydroxyadenine (spectra not shown).

Elementary analysis

A comprehensive elementary analysis was handicapped because of the poor solubility of the examined stone material. Flame emission spectroscopy and electron microprobe examination turned out to be helpful in that nitrogen was found to be a major component, in addition to carbon, oxygen and hydrogen, thus confirming the analogy to purine derivatives. The presence of phosphorus and sulfur as well as that of cations could be excluded.

X-ray diffraction

X-ray diffraction analysis of pulverized material of all 15 samples resulted in essentially identical diffractograms showing two strong reflections at $2\Theta = 10.6°$ and at $27.9°$ and some additional reflections of minor intensity (Fig. 3). Diffractograms of individual samples differed simply by acuity and absolute intensity of their signals. Broad signals of low intensity, as observed in most samples, were apparently due to poor crystallinity. All examined concrements were found to be free of additional crystalline components. Only one sample was identified as being blended with some whewellite.

There was also no fit of the unknown substance to any of the known components of urinary stones for x-ray diffractograms. Even by screening the data bank of the Powder Diffraction File of the International Centre for Diffraction, no corresponding diagram could be detected. However, there was again an apparent similarity to the diffraction pattern of purine derivatives like xanthine and 2,8-dihydroxyadenine.

Crystal and molecular structure

Given the spectral similarity to purine derivatives, the unknown substance was assumed to be closely related to

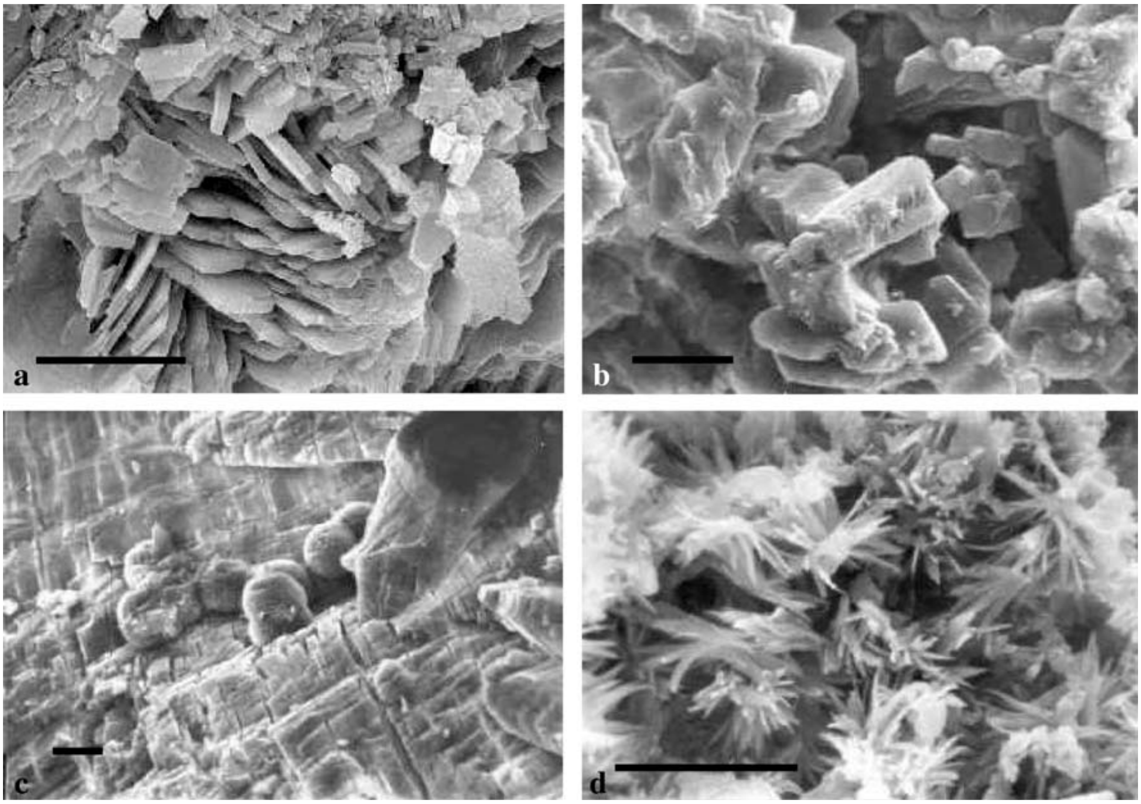

Fig. 1 Scanning electron micrographs of urinary calculi. **a** The new uric acid monohydrate (3,250×), **b** uric acid (2,000×), **c** uric acid dihydrate (1,000×), **d** ammonium hydrogen urate (4,000×). *Bar* = 10 μm

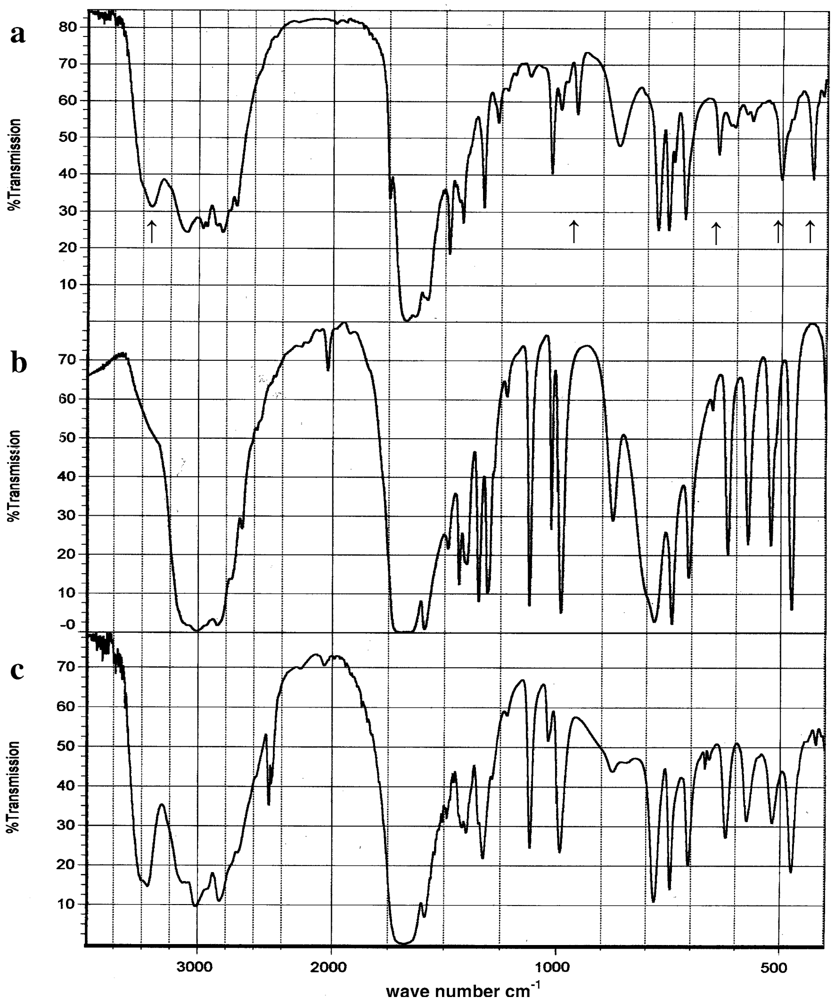

Fig. 2 FT-IR spectra of: **a** uric acid monohydrate, **b** uric acid, and **c** uric acid dihydrate. The H_2O signal at 3,340 cm^{-1} is conspicuous in the uric acid monohydrate spectrogram (**a**) but less intense than for uric acid dihydrate (**c**). The monohydrate spectrum contains additional bands at 435, 505, 646 und 957 cm^{-1}. Moreover, signals in the area 1,250–1,500 cm^{-1} in the monohydrate pattern differ by width and relative intensity from that of the other forms of uric acid

the uric acid molecule. The definite identification of its molecular nature was finally approached by crystal structure analysis.

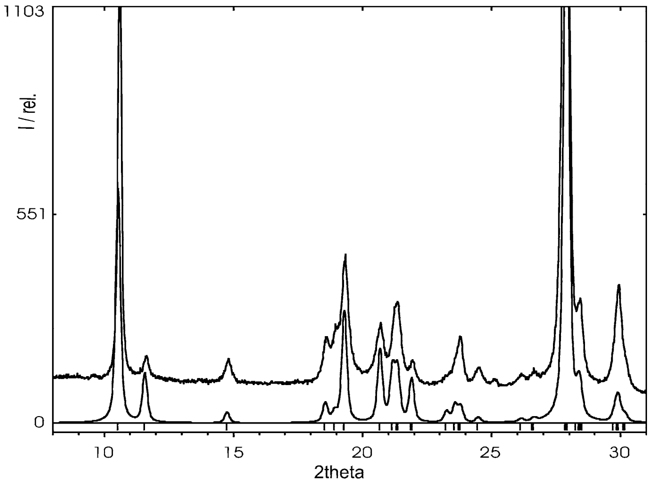

Fig. 3 Experimental powder diffraction diagram (*above*) of one sample of unknown urinary stone material and calculated powder diffraction diagram (*below*) based on the determined crystal structure of uric acid monohydrate

Since sizable single crystals were not available, powder diffraction patterns had to be used for the determination of the lattice constants and of the crystal symmetry. It was found that the structure is monoclinic with the space group $P2_1/c$. The lattice constants are given in Table 1 in comparison with those of uric acid and uric acid dihydrate. The powder diffraction parameters (diffraction angle, d-values and Laue indices) in the range $10° \leq 2\Theta \leq 30°$ are given in Table 2. These can be used for the identification of uric acid monohydrate.

From relations (see Table 1) between the determined cell volume of the unknown substance and that of anhydrous uric acid [8] and of uric acid dihydrate [9], the uric acid molecule was considered as a suitable search model. The correctness of this assumption was proven later by the results of the structure analysis.

The fact that the monoclinic angle is very near to 90° gave rise to additional difficulties in the structural analysis since all (hkl) and (h-kl) reflections are systematically overlapped. Nevertheless, after the first extraction of the integral intensities of 186 single reflections from the powder pattern in the range $10° \leq 2\Theta \leq 60°$ using the IPAP method (intensity partition by approximated Patterson functions [7] incorporated in Powder Cell [3])

Table 1 Crystal data and lattice constants of the pseudo-polymorphic forms of uric acid

	Uric acid monohydrate (this work)	Uric acid [8]	Uric acid dihydrate [9]
Formula	$C_5H_4N_4O_3 \times H_2O$	$C_5H_4N_4O_3$	$C_5H_4N_4O_3 \times 2H_2O$
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/a$	$P2_1/c$
a (Å)	4.786(4)	14.464	7.237
b (Å)	16.812(6)	7.403	6.363
c (Å)	8.598(5)	6.208	17.449
β (°)	90.13(7)	65.10	90.51
V (Å^3)	691.7(8)	602.9	803.5
Z	4	4	4
D (g cm^{-1})	1.787	1.851	1.688

the molecular replacement method PATSEE [4] was applied in order to determine the position of the search model in the unit cell.

After the location of the molecule in the unit cell, a cavity was recognized in the crystal structure by the program CAVITY [5]. This cavity was large enough to hold one water molecule or one ammonium cation. The position of a corresponding non-hydrogen atom was determined by a difference Fourier synthesis. All attempts to decide between water molecule and ammonium cation by simultaneous thermoanalytical and mass spectrometrical investigations failed because of the existence of an additional amorphous component mentioned below. The final decision could by derived from solid state NMR measurement.

Solid state NMR spectroscopy

The ^{13}C NMR solid state spectrum of the new stone material is compared to others of the uric acid system in Fig. 4. The signals in the region of 90–160 ppm belong to the purine system as known from liquid state ^{13}C NMR of pure uric acid [10]. The CP/MAS spectrum of anhydrous uric acid (not shown here) coincides fairly well with that of the dihydrate shown in Fig. 4c.

Table 2 Experimental parameters for the powder diffraction pattern of uric acid monohydrate (Bragg-Brentano geometry, CuKα-radiation), the calculated interplanar crystal spacings (d-values) and Laue indices (hkl)

No of line	2Θ (°) Diffraction angle	d [Å] spacing	Relative intensity	hkl
1	10.58	8.339	59	0 2 0
2	11.62	7.611	7	0 1 1
3	14.77	5.993	7	0 2 1
4	18.62	4.762	9	1 0 0
5	18.98	4.673	10	0 3 1
6	19.33	4.588	18	1 1 0
7	20.69	4.289	11	0 0 2
8	21.26	4.177	13	0 4 0
9	21.33	4.162	13	0 1 2
10	21.94	4.049	7	$-\underline{1\ 1\ 1}$, 1 1 1
11	23.79	3.737	9	1 2 1
12	24.51	3.629	6	1 3 0
13	26.17	3.402	5	0 3 2
14	26.64	3.344	6	$-\underline{1\ 3\ 1}$, 1 3 1
15	27.89	3.196	100	$-\underline{1\ 0\ 2}$, 1 0 2
16	28.41	3.139	13	−1 1 2
17	29.93	2.983	15	−1 2 2, $\underline{1\ 2\ 2}$

The typical pattern of uric acid and of the dihydrate contains one signal at 98 ppm and one at 138 ppm for the aromatic carbons and three signals more or less resolved between 150 and 160 ppm for the three carbonyl groups. In the case of uric salts, the signal at 138 ppm shifts to a lower field as demonstrated for ammonium urate (Fig. 4a). Thus, the spectrum of the new urinary stone material (Fig. 4b) corresponds to a form of uric acid free of the ammonium ion. Therefore it can be definitely identified as uric acid monohydrate.

However, there is a striking peculiarity in the NMR-spectrum in that an additional intense signal at 29.7 ppm (Fig. 4b) was found in all samples classified as uric acid monohydrate by x-ray diffraction. This signal can be assigned to a long CH-– chain as found, e.g., in fats and fatty acids. The structure of this component is not yet fully clear. So far, there is no evidence for carbonyl functions or olefinic carbons that can be attributed to native fatty acids or their salts.

Refinement of the crystal structure of uric acid monohydrate

The refinement of the atomic coordinates and overall thermal parameters for all atoms have been carried out by many cycles of decomposition procedures applying the actual refined structural model and least squares calculations with SHELX-97 [6] using integral intensities and weighted restraints for intramolecular bond lengths and angles. The details of the structural solution and the final atomic coordinates are listed in Tables 3 and 4.

The crystal structure of uric acid monohydrate is illustrated in Fig. 5. In this structure, uric acid and water molecules are connected with each other by intermolecular NH...O and OH...O hydrogen bonds forming molecular layers parallel to the crystallographic {-1 0 2} plane. This is in significant contrast to the crystal structures of the anhydrous uric acid and uric acid dihydrate which also form urinary stone phases [11]. Their crystal structures were determined by Ringertz [8] and Parkin et al. [9], respectively. In both cases molecules are connected by hydrogen bonds forming 3-dimensional networks.

From the crystal structure of uric acid monohydrate, an x-ray diffraction pattern can be calculated. This theoretical diagram is in perfect agreement with the measured diagram from powder preparation as shown in Fig. 3.

Further details of the crystal structure analysis have been deposited in the Cambridge Crystallographic Data Centre (CCDC) and allocated the deposition number CCDC 191315.

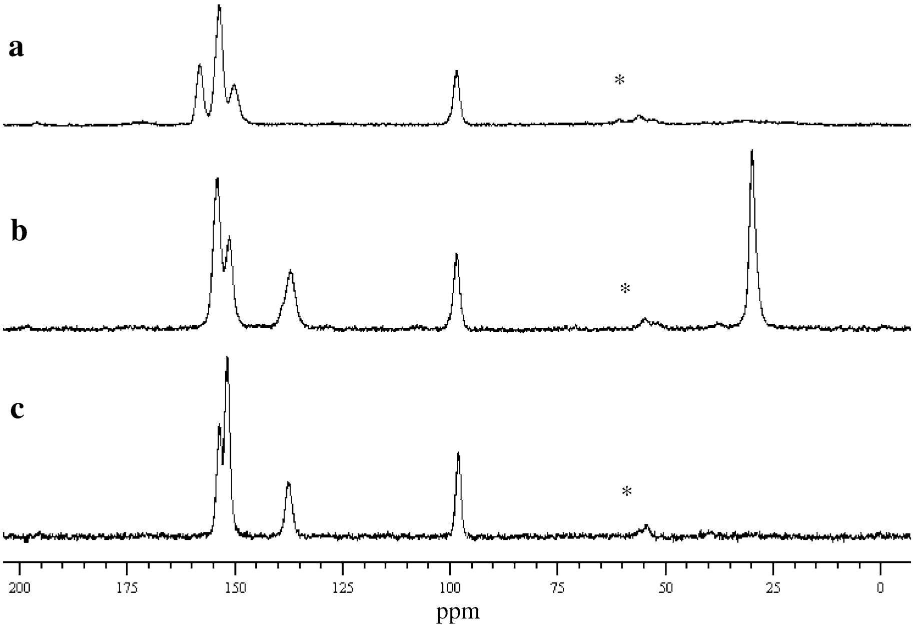

Fig. 4 13 C MAS spectra of: **a** ammonium urate, **b** investigated urinary stones containing uric acid monohydrate, and **c** uric acid dihydrate. (an *asterisk* marks MAS sidebands). The new uric acid monohydrate (**b**) gives a purine spectrum equivalent to both the uric acid and dihydrate (**c**) with signals at 98,138 and between 150 and 160 ppm in contrast to the spectrum of ammonium urate (**a**), where the signal at 138 ppm shifts to lower field. The signal at 29.7 ppm in **b** can be assigned to long CH_2-chain as found, for example, in fats and fatty acids

Discussion

The present study is the first report on uric acid monohydrate representing a genuine crystal state of uric acid. Starting evidence for the existence of this crystal form resulted primarily from the casual observation of an unknown component in urinary concrements amongst a large number of urinary calculi analysed in the routine laboratory. This observation is essentially based on the careful evaluation of x-ray diffractograms and infrared spectrograms backed by many years experience in urinary stone analysis.

Studies into the true nature of this new substance demanded a complex crystallographic approach. This procedure started from the somewhat unexpressive x-ray diffractograms of powder preparations. By a stepwise progress applying extensive calculation and modelling programs, the crystal structure was analysed. Thus, the new component could be identified as uric acid monohydrate ($C_5H_4N_4O_3 \cdot H_2O$). The crucial distinction between the presence of a water molecule or an ammonium ion could finally be deduced from ^{13}C-NMR spectroscopy in favour of water. Definite proof for the correct identification came from the diffraction pattern as calculated from the crystal structure data. This pattern was found to be in perfect match with the measured diffractogram closing thus the chain of evidence.

From its deduced crystal structure, uric acid monohydrate differs profoundly from other crystal states of uric acid. The analysed concrements showed significant differences in morphology and physical features compared to uric acid and its dihydrate form, respectively. Scanning electron micrographs are thus distinctive by presenting a plate-like structure of uric acid monohydrate. By heating the material to 653 K, no changes became apparent neither in the diffraction pattern or in the NMR-spectrogram. The reason for this high stability may be due to strong charge transfer interactions between molecules in adjacent layers being arranged parallel to each other. These interactions are indicated by short distances of 3.30 Å between the molecular planes.

Table 3 Details of crystal structure solution of uric acid monohydrate

Formula	$C_5H_4N_4O_3{*}H_2O$
Formula weight	186.14
Temperature	293(2) K
Wavelength	1.54160 Å
F(000)	384
Crystal size	crystalline powder
Number of single reflections extracted from powder pattern	186
Final R indices [I > 2σ(I)]	R1 = 0.1699, wR2 = 0.3668
R indices (all data)	R1 = 0.1755, wR2 = 0.3702
Largest diff. peak and hole	0.652 and −0.982 e.Å^{-3}

Table 4 Atomic coordinates in the unit cell of uric acid monohydrate

Atom	x	y	z
C (1)	0.7522 (5)	0.0821 (9)	0.6238 (7)
C (2)	0.7855 (5)	0.2214 (8)	0.6220 (6)
C (3)	0.5763 (5)	0.2287 (7)	0.5150 (6)
C (4)	0.4410 (2)	0.1614 (9)	0.4551 (13)
C (5)	0.7170 (3)	0.3510 (8)	0.5699 (14)
N (1)	0.5410 (2)	0.0917 (8)	0.5155 (11)
N (2)	0.8760 (2)	0.1500 (8)	0.6772 (13)
N (3)	0.5350 (2)	0.3083 (8)	0.4834 (13
N (4)	0.8690 (2)	0.2965 (9)	0.6542 (12)
O (1)	0.8100 (3)	0.0139 (8)	0.6623 (14)
O (2)	0.2550 (2)	0.1626 (13)	0.3610 (14)
O (3)	0.7430 (3)	0.4226 (8)	0.5738 (16)
OW	0.2260 (4)	0.4261 (12)	0.3850 (7)

Analogous interactions are not observed in crystals of uric acid and uric acid dihydrate, which are characterized by a fish bone-like arrangement of molecules and lower crystalline stability.

The possible occurrence of uric acid monohydrate as component of urinary stones has to be taken into consideration in the medical laboratory. The evaluation of infrared spectroscopy as the main analytical procedure demands for highly experienced laboratory staff to differentiate uric acid monohydrate from other concremental forms of uric acid. The x-ray diffractograms of the three forms of uric acid show more distinct differences (Fig. 6). Particularly the signals at 10.58° and 19.33° 2Θ, which are unique to uric acid monohydrate, allow for sensitive detection and unambiguous identification. Therefore, x-ray diffraction appears to be the method of choice.

While discussing the occurrence and the generation of concrements from uric acid monohydrate the following observations are pertinent:

1. calculi from uric acid monohydrate are rare
2. the concrements analysed so far are free of other crystalline components
3. all analysed probes contained a sizable share of an amorphous substance of aliphatic character.

Given the infrequent observation of concrements from uric acid monohydrate the crystallization of this form of uric acid may depend on special requirements. Accordingly, the experimental generation of these crystals under laboratory conditions has not been described so far. However, the high fragility of concrements made from uric acid monohydrate may hamper the formation of sizable stone specimens. Thus, gravel-like concrements may be in fact more frequent but may infrequently produce clinical episodes due to their small size.

All concrements of purine derivatives are subjected in our laboratory to x-ray diffraction in addition to infrared spectroscopy. Mixed stones containing uric acid monohydrate are thus unlikely having escaped our attention. Though the sample number is only small in our survey, the predominant state of the monohydrate concrement appears to be that of crystalline purity.

Finally, there remains the somewhat enigmatic amorphous component found in all analysed specimens. Apart from an aliphatic back-bone there is nothing known about its chemical identity. Since this component has constantly been observed in uric acid monohydrate concrements, it may play a crucial role in the crystallization process. Moreover, its high content in respective stones may contribute to their fragility, since

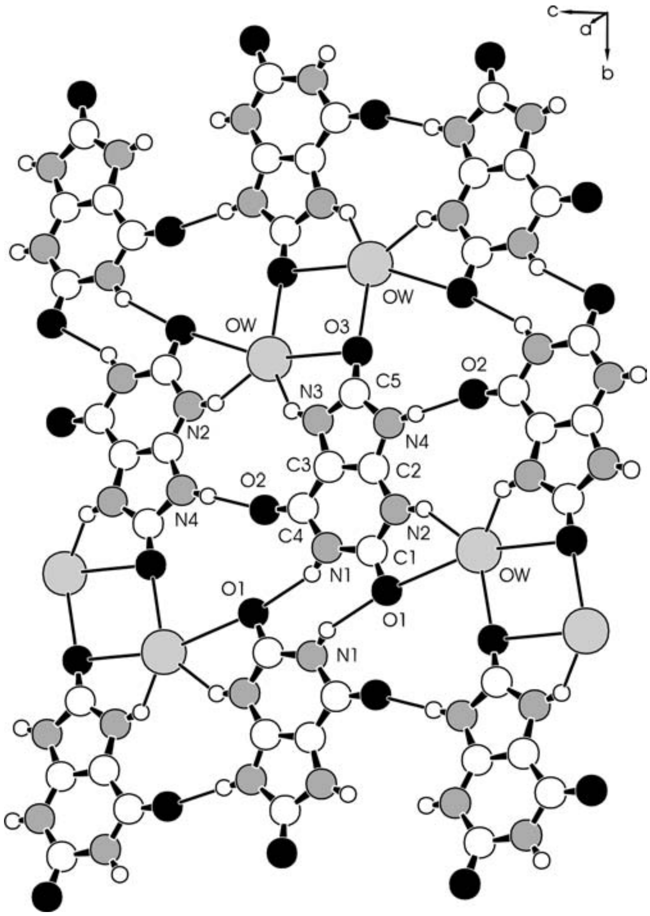

Fig. 5 Crystal structure of uric acid monohydrate. Water and uric acid molecules are connected by hydrogen bonds forming infinite molecular layers (C atoms are *white*, N atoms are *gray*, O atoms are *black*, water molecules are marked *OW*)

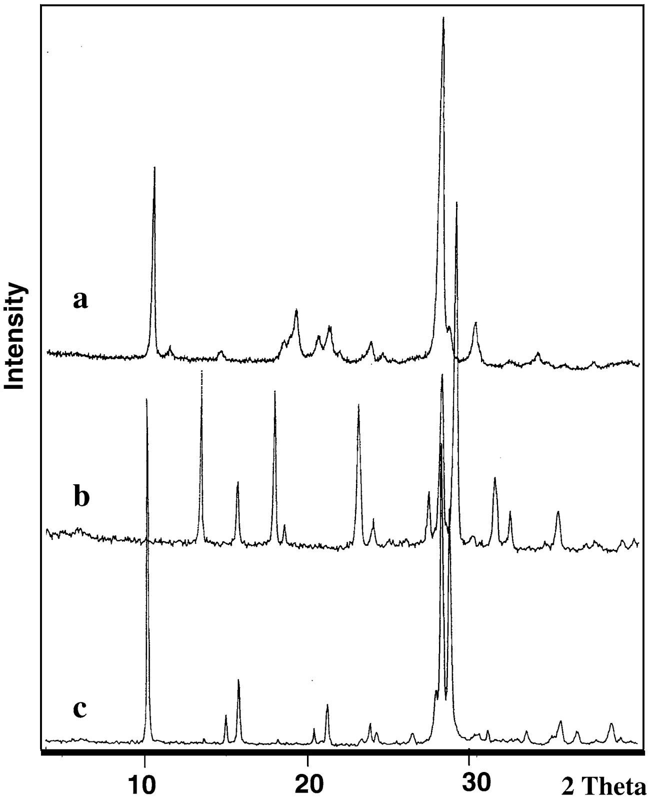

Fig. 6 Powder diffraction diagrams of: **a** uric acid monohydrate, **b** uric acid, and **c** uric acid dihydrate

this fragility is seemingly in contrast to the high stability of the crystal state.

Urinary stones of uric acid and of uric acid dihydrate are prone to formation in acidic urine and are accessible to size reduction by alkalinization. Alkalinization is also an efficient prophylactic measure in preventing reformation. By analogy, the crystallization process of uric acid monohydrate may similarly be favored by an acid milieu in the urine. Once this concremental form is known and occasionally be observed in the routine laboratory, treatment and prophylaxis by influencing urinary pH will have to be tested clinically.

Acknowledgment The authors are grateful to Dr. S. Rogaschewski (Institute of Physics, Humboldt University Berlin,) for the preparation of scanning electron micrographs and to Karin Adam for performing the electron microprobe measurements.

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