UDC 616.6-003.72:544.621

115. Goro Chihara,*1 Nanase Kurosawa,*2 and Etsuji Takasaki*3 : Medical and Biochemical Application of Infrared Absorption Spectra. II.[1] Studies on Urinary Stone by Infrared Spectra. (1).

*(Division of Biochemistry, Central Clinical Laboratory,*1 and Department of Urology,*3 Tokyo University Hospital)*

Clarification of chemical components of urinary calculi is important for the study of the etiology of calculus formation, and for the diagnosis, therapeusis, and prevention of recurrence of urolithiasis.

General chemical components of urinary calculus have been examined by various workers through chemical method,[2] flame analysis, polarizing microscopy,[3,4] and X-ray diffraction,[3,5] and the presence of oxalate, phosphate, uric acid, cystine, etc., has been revealed. It seems that chemical components of urinary calculi have been completely examined.

However, these past methods of analysis were attended with many difficulties and required great deal of effort. Consequently, it was almost impossible to analyse each calculus as it was taken out by surgical operation or expelled naturally. Therefore, these calculi were classified by presumption through external appearance, location, and size.

One of the present authors (G. C.) reported on the analysis of gall stones by infrared spectrum and X-ray diffraction, and it seemed possible to carry out rapid and accurate analyses of urinary calculi by the same methods.

Analysis of urinary calculi by infrared absorption spectra was initiated by Beischer[6] who measured infrared spectra of renal calculi in the region of 4000~650 cm^{-1} by the Nujol mull method and indicated possibility of analysis by this means. There are many different kinds of urinary calculus and many points still remain obscure.

In the present series of work, over 700 infrared spectra were recorded of the surface, internal portion, and nucleus of various urinary calculi (renal, urethral, urinary, cystic, and prostate calculi, and urinary sand), numbering over 200. Of these, only two calculi were of unknown components. By this means, analytical method for urinary calculi was established and this will make it possible to discover a new kind of calculus without going through tedious systematic analyses and to make some congecture as to chemical components of the calculus.

The whole procedure of analysis by the foregoing method requires only 20~30 minutes and, therefore, this method is well suited for clinical assay. The method is now being used in the Central Clinical Laboratory of the Tokyo University Hospital for routine examinations.

*1, *3 Hongo, Tokyo (千原具郎, 高崎悦司).
*2 Present address : Institute of Applied Microbiology, University of Tokyo. Yayoi-cho, Hongo, Bunkyo-ku, Tokyo (黒沢七瀬).

1) Part I : This Bulletin, **6**, 50(1958).
2) A. T. Jensen : Acta Chir. Scand., **84**, 204(1940).
3) E. L. Prien, C. Frondel : J. Urol., **57**, 949(1947).
4) K. Isei : Acta Med. Okayama, **11**, 311(1957).
5) W. J. Smothers, L. H. Siegel : J. Urol., **71**, 647(1957).
6) E. E. Beischer : *Ibid.*, **73**, 653(1955).

Experimental Method

Infrared spectra of urinary calculi were measured by the KBr-pellet method because Nujol mull would conceal important absorptions and because studies on the formation of urinary calculus would require measurement with microquantities of a sample.

Powdered potassium bromide was prepared by pulverization of potassium bromide crystals for prism manufactured by the Institute of Optics, Tokyo Kyoiku University, passed through a 200-mesh sieve, and dried in a vacuum desiccator at 170° for over two days.

The urinary calculi submitted for measurement were those expelled naturally or taken out by surgical operation in the Department of Urology, Tokyo University Hospital, and numbered over 200. The calculi were first submitted to X-ray photography to determine the position of their respective nucleus, each calculus was cut through this nucleus, and samples of approximately 0.5~1.5 mg. in weight were taken from several positions including the nucleus, intermittent layers, and the surface. Each sample was reduced to a fine powder by grinding in an agate mortar for a few minutes. About 150~300 mg. of the above-mentioned powdered potassium bromide was added to this sample in the mortar, mixed thoroughly, and prepared into pellets with a pressure of ca. 100 lb./in^2 under a vacuum of 2~3 mm. Hg. A total of about 700 infrared spectra of urinary calculi were taken with these samples.

The infrared spectrum of a urinary calculus is represented approximately as overlapping of the spectrum of its composite chemical constituents. Consequently, it would be possible to make qualitative and quantitative determinations by preparing standard spectra of each of these chemical components and by selecting a suitable key band. However, in majority of cases, the spectrum of a mixture of solids is not a perfect overlapping of the spectra of each of the components. There were no apparent shifts in the case of urinary calculi and the wave numbers quoted in the present paper should be considered accurate within $\pm 3\,cm^{-1}$, including measurement error.

The standard substances used in the present work were as follows : Calcium oxalate [$Ca(COO)_2 \cdot H_2O$], hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], tricalcium diphosphate [$Ca_3(PO_4)_2$], brushite [$CaHPO_4 \cdot 2H_2O$], calcium hydrogen phosphate [$CaHPO_4$], magnesium hydrogen phosphate [$MgHPO_4 \cdot 3H_2O$], magnesium ammonium phosphate (struvite) [$MgNH_4PO_4 \cdot 6H_2O$], calcium carbonate [$CaCO_3$], uric acid, ammonium hydrogen urate, sodium hydrogen urate, cystine, and xanthine. All these substances were either commercial products or newly synthesized and recrystallized several times, their purity being confirmed through melting point or by X-ray diffraction.

Infrared spectra were measured with Hilger Model H-800 spectrophotometer with NaCl prism and the spectra were corrected with polystyrene. The urinary calculus which is difficult to distinguish in the NaCl region alone (such as the phosphate) was measured in the KBr region.

Results and Discussion

Classification of the infrared spectra of urinary calculi measured as described above revealed that majority of calculi could be roughly classified into seven categories as listed in Table I.

These classifications were made by comparative examination of calculus spectra with absorption of various standard substances. In other words, the spectra of these calculi contained absorptions of all these standard substances. The absorption of standard substances are shown in Fig. 1 and those of the calculi in Fig. 2.

From the examination of these spectra, following conclusions may be drawn.

1) Calcium oxalate calculi consist of a monohydrate and dihydrate. Beischer[6] stated that distinction between them is impossible but it is not impossible. The monohydrate exhibits two weak absorptions at 946 and 880 cm^{-1} while the dihydrate shows one absorption at 913 cm^{-1}. In addition, the monohydrate shows absorption at 650 and around 515 cm^{-1} while the former absorption is absent in the dihydrate and in its stead there is a broad absorption in the region of 600~605 cm^{-1}. The absorption at around 1630 cm^{-1} in the dihydrate is broad and relatively strong.

2) Struvite and hydroxyapatite can be distinguished perfectly. Hydroxyapatite shows strong absorption in the region of 1000~1100 cm^{-1} due to stretching vibration of P-O while struvite shows absorption maximum at 1000 cm^{-1}. This had already been

Table I. Classification of Various Urinary Calculi

1. Calcium oxalate calculi :
   a) Calcium oxalate monohydrate $Ca(COO)_2 \cdot H_2O$
   b) Calcium oxalate dihydrate $Ca(COO)_2 \cdot 2H_2O$
   c) Calcium oxalate (mixed) $Ca(COO)_2 \cdot H_2O + Ca(COO)_2 \cdot 2H_2O$
2. Oxalate-phosphate calculi :
   a) Calcium oxalate-hydroxyapatite $Ca_{10}(PO_4)_6(OH_2)$
   b) Calcium oxalate-struvite $MgNH_4PO_4 \cdot 6H_2O$
   c) Calcium oxalate-hydroxyapatite-struvite
3. Phosphate calculi :
   a) Hydroxyapatite
   b) Hydroxyapatite-struvite
   c) Struvite
   d) Brushite $CaHPO_4 \cdot 2H_2O$
   e) Magnesium hydrogen phosphate trihydrate $MgHPO_4 \cdot 3H_2O$
   f) Magnesium hydrogen phosphate-struvite
4. Urate calculi :
   a) Uric acid
      i) Uric acid (pure)
      ii) Uric acid-hydroxyapatite
      iii) Uric acid-hydroxyapatite-calcium oxalate
   b) Sodium hydrogen urate
      i) Sodium hydrogen urate
      ii) Sodium hydrogen urate-hydroxyapatite
      iii) Sodium hydrogen urate-hydroxyapatite-calcium oxalate
   c) Ammonium hydrogen urate
      i) Ammonium hydrogen urate-calcium oxalate
      ii) Ammonium hydrogen urate-calcium oxalate-hydroxyapatite
5. Cystine calculi :
   a) Cystine (pure)
   b) Cystine-hydroxyapatite
   c) Cystine-struvite
6. Xanthine calculi
7. Protein calculi

pointed out by Beischer[6] but these are not so distinct in the case of a mixture. It would be better to mark the distinction by a strong absorption of struvite at around 1437 cm^{-1} due to NH_4^+ deformation vibration. Struvite shows absorption at 750 cm^{-1} and deformation vibration of PO_4^{3-} appears as only one strong absorption at around 565 cm^{-1}, while apatite has two strong absorptions at 565 and 605 cm^{-1}.

3) Magnesium hydrogen phosphate trihydrate shows four strong absorptions, at 1024, 1060, 1166, and 1238 cm^{-1}, while calcium hydrogen phosphate dihydrate shows four absorptions, at 880, 990, 1050, and 1125 cm^{-1}. These are all characteristic and can be used to distinguish these from other substances. The presence of magnesium hydrogen phosphate in urinary calculi was found for the first time by this experiment. Past chemical methods relied on qualitative reaction of each ion and the presence of this substance could not probably be distinguished when in the presence of struvite, $MgNH_4PO_4 \cdot 6H_2O$.

4) Distinction of the calculi of uric acid system had relied on coloration reaction of uric acid and it was extremely difficult to distinguish between the free acid and sodium or ammonium salts but examination of infrared spectra showed the difference distinctly. It had been known that sodium and ammonium urates were present in urinary calculi but their amount was believed to be very small. However, the present series of experiments have revealed that the calculi containing sodium hydrogen urate amounted to approximately 40% of uric acid calculi.

5) Absorption of cystine was comparatively examined with the result of X-ray

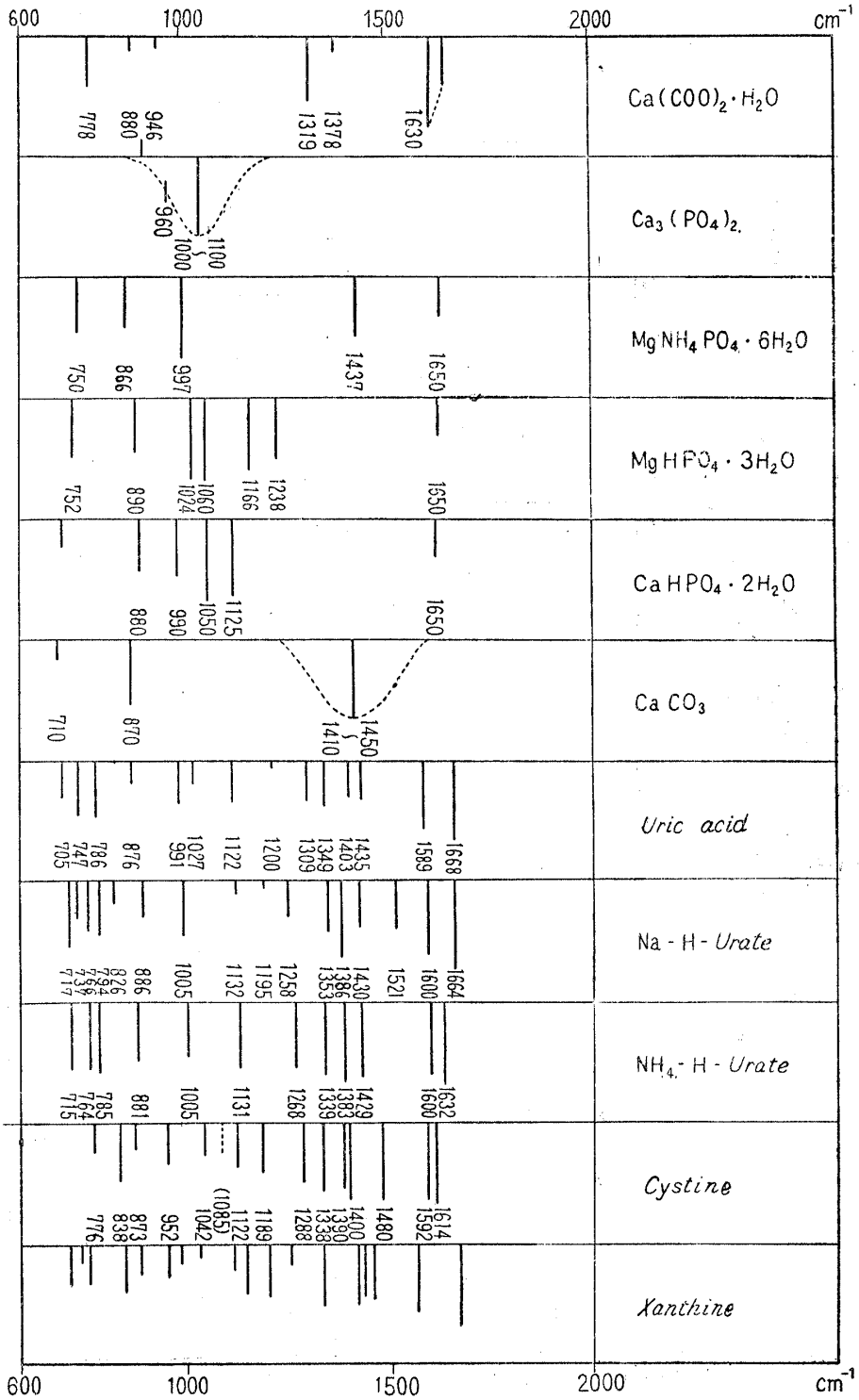

Fig. 1. Infrared Spectra of Components found in Urinary Calculi

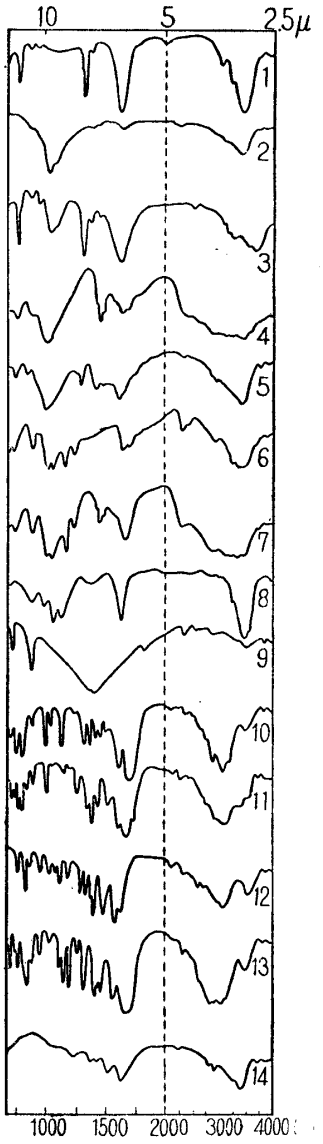

Fig. 2.
Infrared Spectra of Various Urinary Calculi

1. Calcium oxalate
2. Hydroxyapatite
3. Calcium oxalate-hydroxyapatite
4. Struvite
5. Struvite-calcium oxalate
6. Magnesium hydrogen phosphate
7. Struvite-magnesium hydrogen phosphate
8. Calcium hydrogen phospate
9. Calcium carbonate
10. Uric acid
11. Sodium hydrogen urate
12. Cystine
13. Xanthine
14. Protein

diffraction and it was found that the absorption at 1085 cm^{-1} is present only in the crystalline form and not in the amorphous form. As far as the present measurement is concerned, cystine in the urinary calculi is in crystalline form.

6) Proteins are generally present in a minute amount in various urinary calculi and they give strong coloration reaction in a small amount. There are many calculi that contain a large amount of proteins or are composed almost entirely of proteins. Infrared absorption of one of these calculi is shown in Fig. 2.

7) Classification of calculi by components, listed in Table I, did not include calcium carbonate but this substance shows a characteristic absorption at around 1420 cm^{-1}. When a large amount of this substance is present, the absorption at 870 cm^{-1} becomes stronger. This substance is generally present in a small amount in the phosphate calculi but it is still unknown whether it is present in the form of calcium carbonate or in the form of a carbonate-apatite.

8) Organic substances are also present in a minute amount in various calculi and majority of the stones show a very weak absorptions due to C–H stretching and deformation vibrations.

The present series of examinations were always made with due considerations for the result of X-ray diffraction but urinary calculi are likely to be amorphous substances and a much better result can be obtained from infrared spectrophotometer than from X-ray diffraction apparatus, although the use of the latter in conjunction with the infrared photometer is desirable. In the case of a trace of metals, use should be made of flame analysis and other pertinent methods. Quantitative analysis by the KBr-pellet method is now being examined.

Sincere gratitude is expressed to Prof. S. Shibata and Prof. T. Ichikawa for their kind encouragement. The expenses for this work were defrayed by the Grant-in-Aid for Scientific Research from the Ministry of Education which is gratefully acknowledged.

Summary

Analytical method for various urinary calculi through infrared spectral measurement was established.

(Received January 8, 1959)

UDC 547.918 : 582.951.6

116. Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita : Studies on the Constituents of *Digitalis purpurea* L. XIII.*1 On the Diacyl Derivatives of Digitalinum Verum.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.**2)

It has already been reported that deacetylation of the acetate of strospeside,[1] digitalinum verum[1] (I), gitostin,[2] and neogitostin[2] with potassium hydrogen carbonate results invariably in one residual acetyl group in the digitalose portion and a monoacetate is obtained. It had been observed by the present workers that in this deacetylation reaction there is also a reaction product which has not been hydrolyzed to the monoacetate but the formation of such a by-product has not been reported as yet. It has been found in the present series of work that deacylation of some of the digitalinum verum hexaacylates under a suitable condition effected fairly selective deacylation to form 16-acyldigitalinum verum monoacylate.

The objective substance could be obtained in a good yield by decreasing the amount of potassium hydrogen carbonate used in deacylation and by terminating the reaction period at a suitable time. Selection of the reaction time was examined by following the progress of reaction through paper chromatography (Fig. 1).

In the case of acetylated compound, the reaction product was submitted to column partition chromatography through Celite 535, with water-saturated methyl isobutyl ketone as the developing solvent, and needle crystals, m.p. 181~184°, $[\alpha]_D^{26}$ −24°(MeOH)(Fig. 2), were obtained. The product is easily soluble in methanol and ethanol, soluble in acetone and water, and insoluble in ether. Its analytical values and determination of acetyl group agreed with those for digitalinum verum diacetate.

---

*1 Part XII : This Bulletin, **7**, 226(1959).
*2 Hirakawabashi, Sumida-ku, Tokyo (岡野淳二, 傍士和彦, 三木藤作, 坂下昭夫).
1) W. Rittel, A. Hunger, T. Reichstein : Helv. Chim. Acta, **35**, 434(1952).
2) A. Okano : This Bulletin, **6**, 173(1958).