Use of Thermal Gravimetry in the Study of Nephroliths

Thermal gravimetry was used successfully by DUVAL et al.[1] in their attempt to correlate chemical composition with thermal behaviour of approximately 1000 analytical precipitates. Recently, STRATES[2] employed a similar technique in studying the composition and the mechanism of formation of concretions – or precipitates – of biological origin. In the present report, data are presented to demonstrate the use of the thermogravimetric method of analysis in the investigation of nephroliths.

In this study nephroliths were obtained post-operatively from human subjects. Each stone was cleaned externally with a scalpel and washed free of blood, mucus and other extraneous matter. It was then dried at 55 °C in a vacuum and sawn into 2 nearly equal parts with a thin hand saw. One half of the stone was reduced to a fine powder in an agate mortar, whereas the nucleus and the layers of the other half were carefully singled out and ground separately. The powder was dried again in a vacuum and cooled in a desiccator. 100 mg of this powder were weighed each time into platinum crucibles, and thermal diagrams were obtained with an automatically recording Stanton Thermobalance covering room temperatures up to 1000 °C. Using this technique, quite a large number of nephroliths was analysed.

In the Figure A are depicted the differential thermogravimetric (DTG) curves of the nucleus and the surface layers of a representative predominating calcium oxalate nephrolith. Both DTG curves are indeed qualitatively similar to each other. They are also basically the same as curves obtained with analytical precipitates of calcium oxalate[1,2]. The 2 nephrolith curves, however, differ quantitatively in regard to the first – water of hydration – peak. The content in such water of the nuclear portion of the nephrolith is distinctly lower than that of its surface layers.

These data might be interpreted as indicating that (a) the content of the nephrolith nucleus in hydrated calcium oxalate is lower than that of the surrounding layers, or (b) the nuclear calcium oxalate is – primarily or entirely – in the monohydrate form, whereas the surface layers contain the dihydrate. Either $CaC_2O_4 \cdot H_2O$ or $CaC_2O_4 \cdot 2H_2O$ or both have been known to be present in urinary calculi[3,4].

In the Table are given the main lattice spacings derived from an X-ray spectrogram[5] of the powdered half of the nephrolith in question. These values are in good agreement with the *d* spacings for calcium oxalate monohydrate, $CaC_2O_4 \cdot H_2O$, published in the ASTM X-ray card file. Thus, the nucleus and the surface layers of this nephrolith contain the same type of calcium oxalate, i.e. the monohydrate.

In the Figure B are shown the IR-spectra of the nucleus of the nephrolith and its surface layers. In each case 2 mg of the finely powdered specimens were mixed with 200 mg KBr and pressed into pellets. The main wavelengths observed are strong carboxyl absorptions at 6.10 and 7.58 μ with a sharp absorption at 12.73 μ, indicating the presence of oxalate and a somewhat broad but strong OH stretching at approximately 3.0 μ. These values agree fairly well with published IR-data for precipitated calcium oxalate[4,6]. There are also 2 relatively broad but distinct phosphate absorptions at about 9.1 and 9.9 μ and a small carbonate peak at 11.4 μ, indicative of the presence of carbonatoapatite. It is clear that these latter absorptions are deeper and more distinct for the nucleus than for its surrounding layers.

Therefore, the IR-spectra presented here show that the nuclear portion of the nephrolith is richer in phosphate than the surface layers. The distinct nuclear IR carbonate peak, as well as the nearly unchanged CO and CO_2 DTG peaks obtained with the nucleus on the one hand and the surface layers of the nephrolith on the other, indicate that the phosphate is in the form of a carbonated apatite rather than pure – precipitated – hydroxyapatite[7]. This is in agreement with published works of a number of investigators[8–10].

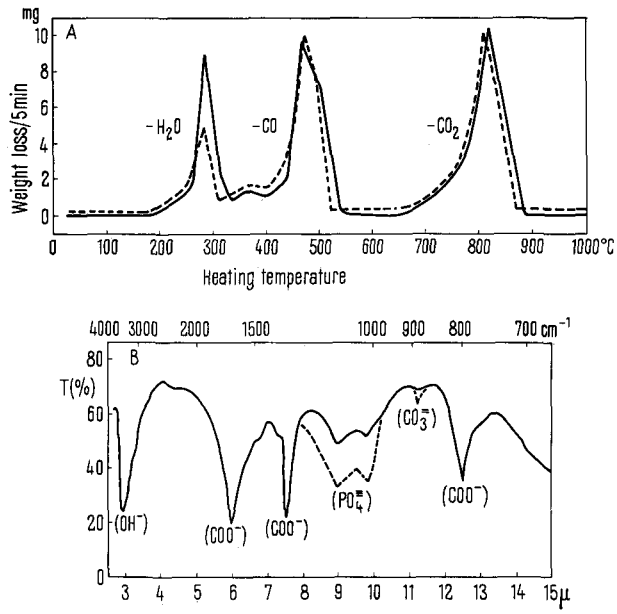

Figure. A = Differential thermogravimetric (DTG) curves of the surface layers (—) and the nucleus (---) of a typical predominating calcium oxalate nephrolith. B = IR-spectra (λ = 2.5–15 μ) of the surface layers (—) and the nucleus (---) of the same nephrolith.

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d-Spacings of a finely-powdered calcium oxalate nephrolith compared with ASTM values for $CaC_2O_4 \cdot H_2O$ (Cu radiation, $\lambda K\alpha$ = 1.542 Å)

d Nephrolith	5.900	3.640	2.960	2.487	2.344	2.246	2.201	2.068
d $CaC_2O_4 \cdot H_2O$ (ASTM)	5.950	3.630	2.969	2.494	2.356	2.256	2.212	2.070

On the basis of these data it is concluded that the interpretation for the thermogravimetrically observed lack of the nuclear portion of the nephrolith in water of hydration is to be found in its comparatively higher phosphate content. From this it follows that the content of the nucleus in oxalate is certainly lower than that of the surface layers.

Additional data on similar lines are now being obtained with the purpose of gaining an insight into the formation mechanism of nephroliths.

Résumé. On examine la possibilité d'emploi de la thermogravimétrie à l'étude des calculs rénaux. On présente des données – radiographiques, thermogravimétriques et spectrophotométriques – pour un calcul composé principalement d'oxalate de calcium. Les résultats spectrophotométriques et thermogravimétriques indiquent que le noyau de ce calcul contient plus de phosphate contenant du carbonate que la surface.

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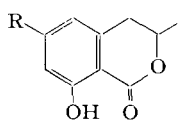
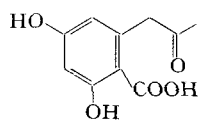
Production of 6-Methoxy-Mellein by *Sporormia bipartis* Cain

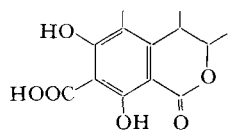
6-Methoxy-mellein (3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin)[1] has been isolated from bitter (stored) carrots[2,3] and in higher yields from carrot root tissue[4,5] inoculated with *Ceratocystis fimbriata, Ceratocystis ulmi, Helminthosporum carbonum* or *Fusarium oxysporum f. lycopersici*. The conclusion was drawn[5] that 'the production of the isocoumarins results from an alteration in the normal metabolism of the carrot root tissue induced either by the presence of fungi, chemicals or environmental conditions'. We have now isolated 6-methoxy-mellein I (R = OCH_3)[6] from submerged cultures of *Sporormia bipartis* Cain. This fungus was grown at 27 °C on a medium consisting of 14 g Difco casamino acids, 24 g glucose, 2.7 g KH_2PO_4, 1.2 g $MgSO_4 \cdot 7H_2O$, 28 mg $FeSO_4$, 3 mg $ZnSO_4$ and demineralized water up to 1000 ml.



I



II



III

6-Methoxy-mellein is one of the numerous fungal isocoumarins related to C-acetyl-*o*-orsellinic acid[7] $C_{10}H_{10}O_5$ (II), which is believed to be biosynthesized by head-to-tail condensation of 1 acetate and 4 malonate units. To the group of isocoumarins with a C_{10}-backbone belong 3-methyl-8-hydroxy-isocoumarin[8] from *Marasmius ramealis*, reticulol[9] (3-methyl-6,8-dihydroxy-7-methoxy-isocoumarin) from *Streptomyces rubrireticuli*, mellein[10] (= ochracin) I (R = H) from *Aspergillus melleus*, resp. *A. ochraceus*, 3-methyl-6,8-dihydroxy-3,4-dihydroisocoumarin[11] I (R = OH) from a mutant of *Aspergillus terreus* and ramulosin[12] (3-methyl-8-hydroxy-3,4,5,10,6,7-hexahydro-isocoumarin) from *Pestalotia ramulosa*. Introduction of additional C_1 units widens the circle of related isocoumarins[13] to include the ochratoxins[14] from *Aspergillus ochraceus*, oospolactone[15], oosponol[16], oospoglycol[17] from *Oospora astringenes*, 4-acetyl-5-methyl-6,8-dihydroxy-isocoumarin[13] from *Aspergillus viridinutans* and dihydrocitrinon[18] (III) from a mutant of *Aspergillus terreus* with three additional C_1 units.

Zusammenfassung. Die Isolierung von 6-Methoxy-mellein I (R = OCH_3) aus Submerskulturen von *Sporormia bipartis* Cain wird beschrieben.

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