Structure Elucidation

Direct Evidence for a Furtive State in the Degradation of Carbasalatum Calcicum**

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The soothing effects of the bark, leaves, and fruits of species of the *Spireæ* genus were known in a mystical, intuitive manner to our most distant ancestors.[1] The knowledge was then formalized, compiled, and propagated throughout the world by the great physicians and pharmacologists, such as Hippocrates, Theophrastus, Dioscorides, and Galen. After this period, this blossoming area of therapeutics lay dormant for almost two thousand years and its renaissance only began in the early eighteenth century when the English pastor and naturalist Edward Stone published his case-study of the effect of willow bark on patients suffering from agues. Novel insights then followed in rapid succession: in 1838 the Italian chemist Rafelle Piria extracted the active component, salicylic acid (SA), in 1874 Hermann Kolbe published its total synthesis, and by 1899 three derivatives devoid of the unpleasant side effects of the pure acid had been discovered. The most useful of these, acetylsalicylic acid (ASA), turned out to be a wonder drug against a broad spectrum of aches: from fever and heart attacks through to inflammations and gout and nowadays is even used to prevent thromboses and embolisms. Recently, salicylates have also been reported to play a role in the reversal of obesity-induced insulin resistance.[2]

However, since ASA is scarcely soluble in water (1 g in 300 ml), it deploys its beneficial effects only after having been attacked by the alkaline media in the duodenal region of the intestine.[3] During these reactions it can cause gastric disturbances in sensitive patients. This can be remedied by elaborating derivatives that are more soluble in water and therefore more easily assimilated in the digestive tract.[3,4] Indeed, improved water solubility and incorporation of biologically active transition metals such as Cu[5] and Zn[6] have often been jointly striven for. To date, about two dozen

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ASA complexes are known. An early commercialized example is carbasalatum calcicum (CC, $[Ca(ASA)_2(urea)]$) which is claimed to be amazingly soluble in water (25%,[4] 231 mg mL^{-1} of solution at 37 °C[7]), unlike other ASA complexes, for example, Cu_2ASA_4.[5] Even though CC has become renowned through the Pharmacopoeia Europea,[8] the details of the solubility and other subtle points regarding its chemistry and physiology are still awaiting elucidation. However, few investigations have been devoted to this elucidation and only a few common analytical results are available. The elemental analysis is consistent with the empirical formula $C_{19}H_{18}CaN_2O_9$. The lines in the IR (KBr) trace ($\tilde{\nu}_h = 1760$ and 1730 (C=0); $\tilde{\nu}_{as} = 1400$ and 1590 (COO); $\tilde{\nu}_s = 1230$ and 1190 (COO) cm^{-1}) compare favorably with those of VO^{2+}/ASA[9] and $[Zn(ASA)_2]\cdot H_2O$[6] and with those given in refs. [10,11]. The $[M+H]^+$ signal of CC cannot be found in FAB mass spectra, probably because Ca forms weak complexes. NMR spectra recorded in D_2O confirm the presence of ASA, but the indications for urea are marginal. All these results do not provide any definite corroboration of the nature of the complex. A solid-state ^{17}O NMR investigation, possibly involving the use of coupling tensors, might offer such an answer but would require huge single crystals and appears to be a formidable task. Since not even tiny crystals are available (the Merck Index, 13th ed., 2001, talks about an amorphous powder as recently as 2001), we undertook a synchrotron powder diffractometry[12] study, which furnished the proof-of-existence of CC through its crystal structure. Furthermore, we present here novel details concerning the degradation of CC in pure water,[3,4,7] namely the isolation and structure of the furtive intermediate species acetylsalicylatum calcicum (AC). The high solubility of CC is also reassessed.

The well-established synthetic procedure[8] yields CC as a white powder containing well-developed square platelets too tiny for single-crystal diffraction studies. Recrystallization of CC showed that a) slow diffusion of various poor solvents into aqueous solutions was rather unsuccessful, except that acetone gave rise to an oddity, namely colorless cuboctahedra that turned out to be ASA,[13] and b) slow evaporation of a supersaturated (> 25%[4,7]) aqueous solution did not afford crystals if the process, which takes a few hours, was allowed to go to completion. Spectroscopic analysis of the residues reveals that the deacetylization[3,4] of ASA is almost complete at this stage.

In view of this chemical evolution of CC in water we decided to scrutinize its behavior in this solvent by optical microscopy. CC was added to water in a Petri dish until the crystals no longer dissolved but floated on the surface of the mother liquor. These undissolved grains of CC could easily be seen on the surface by means of a binocular microscope. Surprisingly, the grains then began to disappear and, successively, two new habitats nucleated in the heart of the mother liquor: thickish joists first and sturdy laths about half a minute later. After about 90 seconds of coexistence, the joists began to disappear, but, with swift action, crystals of this furtive form could be isolated and, as they were stable enough in air, analyzed by X-ray diffraction. They were found to be *catena*-bis(μ_2,η^2-acetylsalicylato)-diaquo-calcium dihydrate (acetylsalicylatum calcicum, AC).[14] Their structure (Figure 1) contains columns (rod group $P(12/c)1$) of edge-sharing CaO_8 polyhedra (approximate point group C_{2v}) embedded in a matrix of acetylsalicylato groups bound by van der Waals

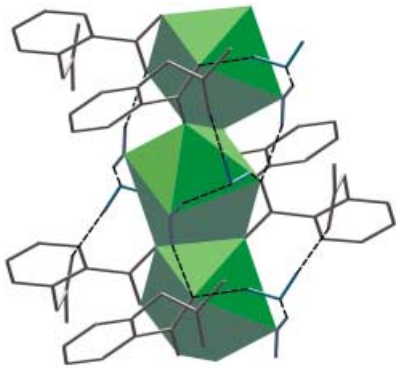

Figure 1. A [001] column of edge-sharing CaO_8 polyhedra (green) in acetylsalicylatum calcicum. Hydrogen bonds (black, dashed) between the bridging acetylsalicylato groups and coordinated (dark blue)/crystalline (turquoise) water molecules are also shown.

forces (shortest H···H contacts: 2.92–3.60 Å). These columns, separated by about 12.5 Å, are stabilized by extensive hydrogen-bonding interactions (normalized bond lengths[15] of 1.77–2.00 Å and angles of 160–166°) between the coordinated water molecules, the crystalline water molecules, and the carbonyl oxygen atoms of the ester groups. A small decay in intensity occurred during the three to four days of data collection,[14] which might arise from the adsorption of some moisture (the crystal surface turned white). This phenomenon is reminiscent of the problem mentioned by Lawrence[3] in his patent. Indeed, he claimed a maximal number of water molecules of 3.5 for a synthesis leading to storable AC. In view of the presence of two molecules of water in our structure, we conject that 1.5 additional water molecules might be at the crystal surface. The laths, once dried, are also stable in air and have been shown to be ASA[13] by single-crystal X-ray diffraction studies.

Since CC is instable in water it was studied by synchrotron powder diffractometry (Figure 2) on beamline BM16 at the European Synchrotron Radiation Facility. The successful solution of this structure (with 31 non-hydrogen atoms) shows that CC is indeed a unique phase,[16] namely *catena*-bis(μ_2,η^2-acetylsalicylato)-μ_2-urea-calcium. CC[16] (Figure 3) contains chains (rod group $P(1)2_1(1)$) of corner-sharing CaO_6 polyhedra (very approximate D_2 symmetry). These columns (separated by $c/2$) are cross-linked along the c axis by way of N−H···O=C bonds (N−O distances of 2.97–3.29 Å) to form semirigid (100) sheets held together by van der Waals forces only along the a axis. The ester groups in both acetylsalicylates are roughly at right angles (84 and 89°) with respect to their benzene rings, whereas the carboxylate groups form angles of 32 and 35° with the latter. For the major part, the freely refined distances in CC fall well within the bracket of distances (2.293–2.415 Å) found in the CCDC

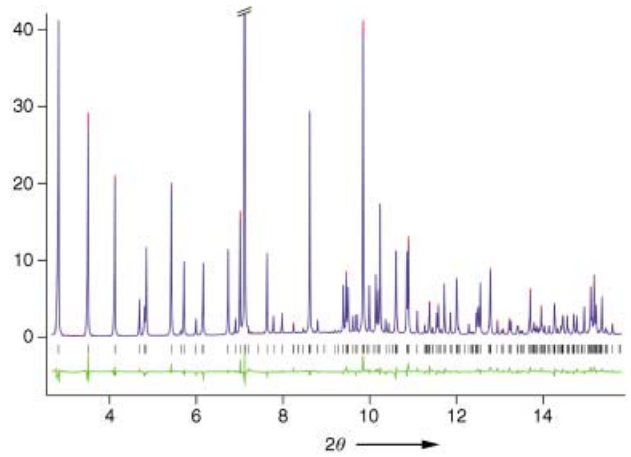

Figure 2. Excerpt from the observed (red), calculated (blue), and difference (green) profiles resulting from the Rietveld refinement for carbasalatum calcicum. The black marks indicate reflection positions.

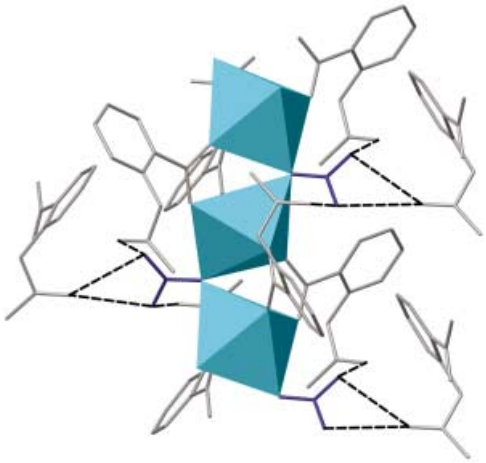

Figure 3. A [010] column of corner-sharing CaO_6 octahedra (turquoise) in carbasalatum calcicum. Hydrogen bonds (black, dashed) between the bridging acetylsalicylato groups and coordinated urea molecules (dark blue) are also shown.

database.[20] Our Ca–O(urea) distances, however, are the longest ones reported (2.503(6) and 2.519(6) Å). The extreme values in CC might arise from the fact that the urea molecule reconciles several roles, namely bridging the two CaO_6 octahedra, participating in hydrogen-bonding interactions with the acetyl ester moiety, and optimally embedding itself in the general packing of the structural components. Thus, the Ca–O(urea) bond acquires an almost van der Waals like character which we believe to play an essential role in the solvation behavior of CC. We should like to emphasize that this uncommon crystallization sequence could not have been established without the help of the human eye, much as in the recent report[21] about concomitant polymorphs. Indeed, an attempt to corroborate the degradation of CC in a heat-flow calorimeter coupled with a focused-beam reflectance-measurement probe failed;[22,23] no optical or thermal signal of the intermediate AC could be detected.

Finally, we endeavor to explain the various phenomena observed while crystallizing CC. It appears that in a supersaturated aqueous solution, coordinated urea in CC rapidly becomes unstable and is replaced by two water molecules. This instability might be indicated by the particularly long Ca–O(urea) distances. This hypothesis is further endorsed by the complexation constants of the exchange $Ca^{2+} + 2\,\text{urea} \rightarrow Ca(\text{urea})_2$ for which $\log\beta_2$ is -0.6 in 4 M $NaClO_4$.[24] The exchange is reinforced by the large number of potential hydrogen bonds with the water molecules. However, the thus-formed AC does not survive for a long time either and the acetylsalicylato groups are quickly replaced by water molecules. The high solubility of CC in water is definitely relegated to the realm of myth and CC therefore joins the ranks of other ASA complexes. Indeed, with the Ca^{2+} ion being a hard acid and H_2O being a hard base, the dissolved species is most likely $[Ca(H_2O)_6]^{2+}\cdot 2\,\text{ASA}$.

We have added two pharmaceutically important structures to the class of complexes containing ASA (until now only the structure of $[Cu^{II}(ASA)_2]$[25] had been reported). Besides the mere knowledge of the molecular arrangement in CC and AC, the careful observation of the solvation behavior of CC has provided us with a better understanding of certain crucial features of this complicated quaternary system. Finally, this study has confirmed the value of a simple, regretfully neglected, tool, namely careful observation of crystals with a microscope, which has led to the detection, isolation, and characterization of a furtive species.

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