On the Formation of Whewellite in the Cactaceae Species *Opuntia microdasys*

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The isolation of well formed crystals of the biomineral whewellite (monohydrated calcium oxalate) from *Opuntia microdasys*, a cactaceae species found in central Mexico, is described. The morphology of the crystals was investigated by means of electron microscopy. Infrared spectroscopic measurements allow an unambiguous characterization of the nature of the crystals. This is the first report of the presence of a biomineral in this species.

In relation to our studies on biomineral formation in different plant species, we have recently reported the presence of weddellite (calcium oxalate dihydrate) in *Chamaecereus silvestrii*, a cactaceae species original from northern Argentina (Monje and Baran, 1996). In the present paper we report a similar investigation, performed on another cactaceae species, *Opuntia microdasys*, originally found in different areas of central Mexico (Backeberg, 1983).

The study was performed with commercially available samples of *Opuntia microdasys* (Lehm.) Pfeiff.v.pallida. Abundant crystalline material could be separated from the plant tissue, as described in the experimental part. Its microscopic observation revealed the presence of well formed, practically isodiametric druses. Further observations, using scanning electron microscopy, showed a certain degree of damage of the crystallites, probably introduced during the isolation process. The obtained druses are constituted by aggregates of hundreds of small crystals showing a star-like form, with very thin and acute points, and which appear to grow out from the center of the druse, as shown in Fig. 1.

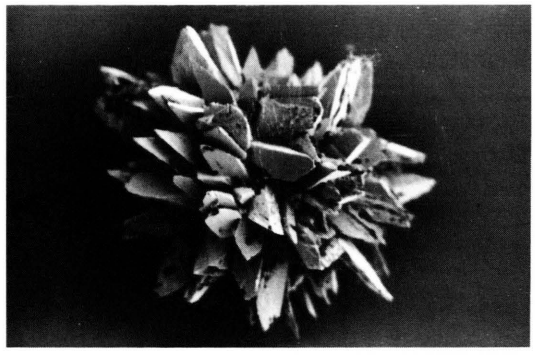

Fig. 1. SEM photography showing the crystal druses of *Opuntia microdasys* (156 x magnification).

The infrared spectra of samples obtained in three different and independent isolation experiments were identical and allow to identify the crystalline material as whewellite (monohydrated calcium oxalate, $CaC_2O_4 \cdot H_2O$) by comparison with literature data (Babic-Ivancic *et al.*, 1985; Varetti and Volponi, 1995). One of the obtained spectra is shown in Fig. 2.

This spectrum appears very well defined and free from spurious bands, showing the high purity of the biomineral. The two stretching vibrations of the carboxylate groups appear as very strong infrared bands. The antisymmetric stretching is located at 1622 cm^{-1}, whereas the corresponding symmetric mode is seen at 1317 cm^{-1} and presents a weak doublet (1381 and 1365 cm^{-1}) on its higher frequency side. The vibrations of the water molecule are nicely resolved. In the region of the O-H stretching vibrations four bands (3485, 3429, 3340 and 3059 cm^{-1}) are clearly seen. The remaining band in this region, found at 3261 cm^{-1} has been assigned to the first overtone of the $\delta(H_2O)$ mode (Babic-Ivancic *et al.*, 1985), whereas the fundamental is evidently overlapped by the strong 1622 cm^{-1} band. Besides, four librational modes of water could be identified at 949, 885, 663 and 596 cm^{-1}. The other two strong bands, found at 781 and 517 cm^{-1} may be assigned to OCO deformational vibrations, coupled to some extent with υ(C-C) modes (Nakamoto, 1978). The great splitting

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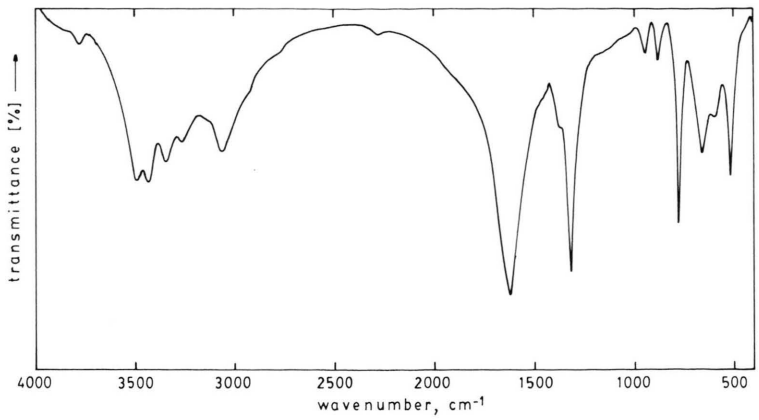

Fig. 2. Infrared spectrum of whewellite isolated from *Opuntia microdasys*.

on the υ(OH) region and the appearance of an important number of bands assignable to librational modes of water may be derived from the complex characteristics of the hydrogen bonds present in this lattice (Deganello and Piro, 1981).

Previous studies on other species of the *Opuntia* family, i.e. *Opuntia imbricata* (Haw.) and *Opuntia englemanii* (Parry) also shows the presence of whewellite crystals, with a similar morphology as that found in the present study (Rivera and Smith, 1979). This opens the interesting question whether the formation of the monohydrated calcium oxalate is characteristic of this cactaceae family.

Experimental

Small stems of *Opuntia microdasys*, commercially available, were cultivated in the laboratory, under natural illumination at room temperature (20 °C) and irrigated with the usual water regimen which favors the adequate development of this type of plants. After a growing of ca. five months the stems attain an adequate length for the crystal extraction.

For this extraction, the most mature part of the vegetal tissue, corresponding to the basal stem halves, were selected and thoroughly washed with distilled water. The fine thistles and the epidermis were separated and the remaining material was washed again. Small cutted portions of these tissues were incubated in a small volume of an acetic acid solution (40% v/v), at room temperature. After ca. 48 hs., the caulinar tissue appears widely degraded and the crystals liberated to the acid solution, in which the calcium oxalates are totally insoluble (Dormer, 1961). The crystals could be separated manually, without difficulties. They were washed four or five times with bidistilled water and submitted to microscopic and spectroscopic analyses. The described technique, used instead the enzymatic procedure employed for the isolation of weddellite crystals from *Chamaecereus silvestrii* (Monje and Baran, 1996) also demonstrated a great effectivity and, although it requires a somewhat longer operation time, it is much more cheaper.

Moreover, we could isolate crystalline samples from dried plant material. In this case, the removed stems were allowed to dry in air. After removement of thistles and cuticle, the remaining dry material was placed in 12-cm Petri dishes and covered with water. The soft material was macerated with dissection knives, which freed the crystals from their respective cells. They were manually collected and washed several times with bidistilled water. This separation process is also very effective and faster than that described above.

Scanning electronic microscopy was carried out with a JEOL 35 CF instrument (Jeol Co., Inc., Japan) at an acceleration voltage of 5 kV. The crystals were mounted on an adhesive conducting aluminium tape and coated with gold, in the usual way.

The infrared spectra were obtained by means of a Bruker IFS 66 spectrophotometer in the spectral range between 4000 and 400 cm^{-1}, using the KBr-pellet technique (ca. 4 mg of the powdered crystal sample dispersed in 100 mg of KBr).

Acknowledgements

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