

Evidence of formation of glushinskite as a biomineral in a Cactaceae species

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Abstract

The X-ray diffractometric and infrared spectroscopic investigation of crystalline material isolated from the Cactaceae species *Opuntia ellisiana* shows the presence of a very complex mineral composition, including whewellite (monohydrated calcium oxalate), opal (SiO_2), calcite (CaCO_3) and glushinskite (dihydrated magnesium oxalate). This is the first report of the presence of magnesium oxalate in plants.

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1. Introduction

Mineral deposits in plants have been known since the seventeenth century but only in the last 50 years has their wide distribution in the vegetal kingdom been definitively confirmed. However, a complete understanding of the distribution, development, and especially the physiological significance, of these mineral deposits has not yet been achieved (Monje and Baran, 2004a). Three biomineral types are especially common and widely distributed in plants: calcium oxalates, calcium carbonates and silicon dioxides (Arnott, 1982; Monje and Baran, 2004a).

Some higher plants may accumulate enormous quantities of inorganic material, and this is especially true for some members of the Cactaceae family (Franceschi and Horner, 1980; Monje and Baran, 2002, 2004a). For example, as early as in 1938, a cactus species (*Cactus*

senilis) was described as containing about 85% of its dry weight as calcium oxalate (Cheavin, 1938). Calcium oxalate occurs in two different hydration states in plants, either as the monohydrate (whewellite) or as the dihydrate (weddellite) (Arnott, 1982; Monje and Baran, 2004a).

In a recent study, we have investigated the characteristics of this biomineral in members of two of the main traditional subfamilies of the Cactaceae family (Opuntioideae and Cereoideae) and found that the first one always synthesizes whewellite; whereas, in the second one, only weddellite deposits were found. Coexistence of the two types of oxalates could never be demonstrated in any of the so far investigated species, suggesting a definite but different genetic control (Monje and Baran, 2002). In certain cases, we have also found the presence of some other biominerals in addition to calcium oxalate, for example α -quartz (Monje and Baran, 2000) or opal and calcite (Monje and Baran, 2004b).

During the investigation of biomineral samples isolated from the Cactaceae species *Opuntia ellisiana* we have now found an even more complex biomineralization

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pattern. In this case, together with whewellite, opal and calcite, the presence of glushinskite, dihydrated magnesium oxalate ($\text{MgC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), could clearly be established.

2. Results and discussion

A first IR spectroscopic investigation of the crystalline sand isolated from a sample of *O. ellisiana*, immediately showed an unexpected spectral complexity suggesting the presence of whewellite and biogenic silica (opal), together with some other materials. One of them could be easily identified as calcite, by comparison of this spectrum with those recently recorded from a series of biomineral samples, isolated from some other Cactaceae species also belonging to the Opuntioideae subfamily, in which the presence of CaCO_3 has been clearly established (Monje and Baran, 2004b).

A greater amount of the biogenic material was collected and submitted to X-ray powder diffractometry for the complete identification of all the components of the biomineral mixture. Fig. 1 shows one of the obtained powder diffractograms. Its analysis clearly confirmed the presence of whewellite, opal and calcite and also revealed the presence of glushinskite.

On the basis of the relative intensities of the diffraction peaks it can be concluded that whewellite is the most abundant component of the biomineral mixture. The unexpectedly high intensity of the diffraction peak at about 30° originates by the superposition of the most intense calcite reflection with one medium intensity whewellite peak. Notwithstanding, most of the calcite diffraction peaks appear only with a very weak intensity. This behavior is in agreement with the fact that usually calcium carbonate found in plants is amorphous calcite (Arnott, 1982).

The relative abundance of opal is also difficult to establish, because typically biogenic opal only produces very broad and scarcely defined X-ray diffraction peaks.

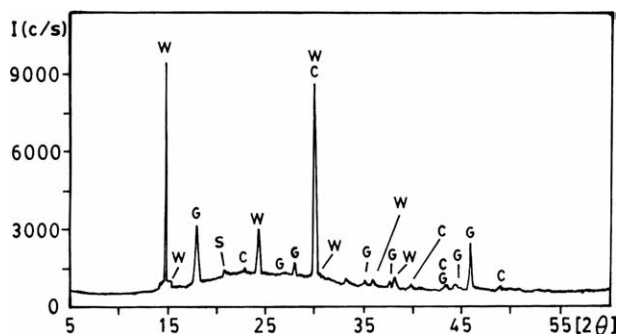


Fig. 1. X-ray diffraction pattern of the crystalline sand isolated from a sample of *O. ellisiana* (C, calcite; G, glushinskite; S, opal; W, whewellite).

The broad and undefined feature in the 2θ -range between 15° and 30° observed in Fig. 1, is characteristic of the diffraction pattern of biogenic opals (Kamatani, 1971; Lanning et al., 1958).

The presence of the four mentioned biominerals could be unambiguously confirmed by means of the infrared spectrum of the crystalline sand. One of the obtained spectra is shown in Fig. 2 and its analysis presented in Table 1. The antisymmetric stretching vibration of the oxalate group of glushinskite presents a doublet structure ($1664/1641\text{ cm}^{-1}$) identical to that found in the synthetic material. The corresponding motion of whewellite is partially overlapped by this doublet and can only be seen as a weak shoulder (ca. 1620 cm^{-1}). Regarding the symmetric stretchings of the anion, that corresponding to whewellite is evidently overlapped by one of the components of glushinskite (band at 1323 cm^{-1}). The other component of the glushinskite doublet (1373 cm^{-1}) is found at the same position and with the same relative intensity as in the synthetic compound. Some other characteristic whewellite and glushinskite absorptions could also be clearly identified (cf. Table 1).

Two of the typical IR bands of opal could be identified. One of them is the very strong and relatively broad band centered at 1050 cm^{-1} , and related to a Si–O stretching motion. The other one, related to SiO_4 -bendings, appears as a medium intensity band at 781 cm^{-1} , presenting a similar shape as in other samples of biogenic silicas (Kamatani, 1971), although also pure whewellite presents a IR band in this same region. A third band usually found in these biominerals in the spectral range between 460 and 470 cm^{-1} , is surely immersed in the strong absorption block of bands located between 750 and 430 cm^{-1} .

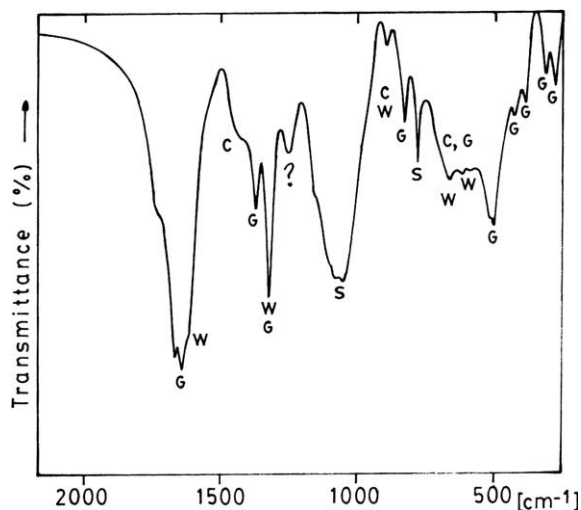


Fig. 2. FTIR spectrum of the crystalline sand isolated from a sample of *O. ellisiana* in the spectral range between 2000 and 400 cm^{-1} (C, calcite; G, glushinskite; S, opal; W, whewellite).

Table 1
Analysis of the IR spectrum of the biomineral isolated from an *O. ellisiana* sample in the spectral range between 2000 and 400 cm^{-1}

Plant material	Whewellite ^a	Calcite ^b	Glushinskite ^c	Opal ^d
1718 sh				
1664 vs			1664 vs	
1641 vs			1641 vs	
1620 sh	1622 vs			
1430 m, br		1432 vs		
1373 m			1373 m	
1323 vs	1317 vs		1326 vs	
1246 m				
1050 vs				1100–1080 vs
891 w	885 w	874 s		
829 m			830 m	
781 m	780 s			800–780 m
700 sh		712 m	693 m	
665 w	663 m			
588 w	596 w			
501 s			500 s	
435 w			422 m	
388 w			386 m	
320 m, 275 m			318 m, 274 m	

Band intensities: vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; br, broad.

^a Monje and Baran (1997).

^b Ross (1972).

^c This paper.

^d Kamatani (1971).

The presence of calcite is not easy to confirm from analysis of the IR spectra. The antisymmetric carbonate-stretching mode is only present as a relatively broad feature at about 1430 cm^{-1} , at the higher energy side of the oxalate doublet at 1373/1323 cm^{-1} . The out-of-plane deformational mode is probably overlapped with the whewellite band at 891 cm^{-1} , whereas the in-plane deformation is only insinuated as a shoulder at about 700 cm^{-1} . Notwithstanding, this spectroscopic behavior closely resembles that previously found for calcite in the biomineral sand of other members of the Opuntioideae subfamily (Monje and Baran, 2004b).

All the investigated spectra present another very broad and strong band, not shown in Fig. 2, centered at about 3410 cm^{-1} and unambiguously related to the stretching vibrations of the water present in whewellite and glushinskite and to the SiO–H stretchings of opal. The corresponding deformational mode of water, usually found at around 1600 cm^{-1} (Ross, 1972) is overlapped by the strong 1664/1641 cm^{-1} doublet.

In all of the recorded spectra, an additional band (marked with the symbol ? in Fig. 2) located at 1246 cm^{-1} was observed. This band could not be assigned to any of the four identified biominerals or to another, different, species. Interestingly, in the previously investigated biominerals a similar IR band was always found between 1240 and 1250 cm^{-1} (Monje and Baran, 2004b).

The obtained results present some aspects of special interest in relation to biominerals and biomineralization processes in plants. They constitute a new example of the generation of whewellite in a member of the Opuntioideae subfamily (Monje and Baran, 2002) and show again the presence of opal and calcite as biominerals in a Cactaceae species (Monje and Baran, 2004b). Notwithstanding, the most interesting aspect of this study is the finding of glushinskite in a plant, as the presence of this compound has so far been never reported as a biomineral in any botanical species.

As a result of the previously investigated calcium oxalates from the main traditional subfamilies of the Cactaceae family, we suggested a definite genetic control for the deposition of the biomineral in these species (Monje and Baran, 2002). As we could so far not establish the presence of glushinskite in other members neither of the Opuntioideae subfamily nor in any other Cactaceae, it is obvious that today it is impossible to establish if also the deposition of this magnesium oxalate responds to such a type of control. On the other hand, it is also not yet possible to speculate about the importance of this finding in relation to evolutionary aspects among these plant species.

A detailed literature search showed that different metallic oxalates are generated naturally by reaction of oxalic acid, secreted by some lichens, with the minerals present in the rocks in which they develop. There is a close relationship between the chemical composition of the substratum and the type of insoluble oxalate accumulating immediately beneath or on within the thallus (Adamo and Violante, 2000). In the Grampian Region (Scotland) an outcrop of serpentinite, a rock consisting almost entirely of magnesium silicate minerals with very low calcium content, appreciable amounts of glushinskite were found in thallus of *Lecanora atra* as well as at the rock-lichen interface (Wilson et al., 1980). It has also been suggested, that in these natural minerals, magnesium could be substituted by Ni(II), Co(II), Fe(II), Zn(II) and Mn(II) in the glushinskite structure, as all the dihydrated oxalates of these cations are isomorphous (Wilson et al., 1980, 1981). These are so far the unique reports of naturally generated glushinskite, by interaction of living organisms with the mineral surrounding. Therefore, the finding communicated in this paper on the presence of $\text{MgC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ in a Cactaceae species, seems to be the first report of the presence of this biomineral in the plant kingdom.

3. Experimental

Samples of *O. ellisiana*, from Chihuahua (Mexico) were kindly provided by Dr. David Eppele (Director, Arizona Cactus and Succulent Research; Bisbee, Arizona).

The crystalline material was directly isolated from the dried plants. After removal of thistle and cuticle, the remaining dry material was placed in 12-cm Petri dishes and covered with EtOH–H₂O (96:4). The soft tissue was macerated with dissection knives, which freed the crystalline material from their respective cells. This material was present mainly as crystalline sand, which was manually collected and washed several times with EtOH.

A sample of synthetic glushinskite, MgC₂O₄ · 2H₂O, prepared for comparative purposes, was obtained by reaction of an excess of ammonium oxalate with 1.0 M magnesium chloride at boiling temperature, over a water bath. The microcrystalline precipitate was removed by filtration, washed several times with hot water and left to air dry. Its powder diagram confirmed the generation of the crystalline modification known as β-MgC₂O₄ · 2H₂O (Walter-Lévy and Perrotey, 1970).

X-ray powder diagrams were measured with a Philips PW 1710 diffractometer, using Cu–Kα-radiation, and operating at 45 kV and 30 mA.

The infrared spectra were recorded on a FTIR-Bruker IF66 spectrophotometer in the spectral range between 4000 and 400 cm^{−1}, using the KBr pellet technique (ca. 4 mg of powdered crystal sand dispersed in 100 mg of KBr). The spectra were recorded with material extracted from four different samples, showing excellent reproducibility.

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