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Characterization of a mineral coating of the plant Dyerophytum indicum

Abstract: The mineral coating of Dyerophytum indicum Kuntze (Plumbaginaceae) was analyzed by isotope ratio mass spectrometry, X-ray diffraction, thermogravimetric and differential thermal analysis, and scanning electron microscopy/energy-dispersive X-ray spectroscopy methods. Its composition was found to be similar to those of the carbonate mixtures isolated from rotting cacti and speleothems. The coating consisted of three major phases (monohydrocalcite, nesquehonite, and calcite), a minor phase of hydromagnesite, and traces of silica and sylvite. This is the first time that the occurrence of monohydrocalcite, nesquehonite, and hydromagnesite in a living higher plant has been reported. A possible mechanism of the formation of the coating is also discussed.

Keywords: Dyerophytum; plant biomineralization; Plumbaginaceae.

1 Introduction

Dyerophytum indicum Kuntze (Arabic: ‘mellah’ or salty) belongs to the Plumbaginaceae family. It is an erect shrub up to 2 m tall, and is distributed throughout the Arabian Peninsula and Western India. In Oman, it is a common plant of wadis (desert and mountain erosion valleys of intermittent streams) at altitudes of up to 1800 m [1].

The leaves of D. indicum are covered with friable white mineral coating (Figure 1) which has been described in the literature as a mere salt, even usable for cooking (see e.g. [1]). However, based on our own experience in the field, the coating is tasteless, insoluble in water, and contains carbonates. The apparent discrepancy of the mineral composition with that reported in the literature led us to perform an initial chemical characterization of this coating.

2 Materials and methods

The samples of mineral coating from the leaves of D. indicum were collected from two sites in the Sultanate of Oman: the water-bearing floor of Wadi Muaiden (N23° 0.0′; E57° 39.5′), and from a wadi near Muscat (N23° 27.7′; E58° 20.5′), in December 2010. Average daily temperatures at the time of collection ranged from +15 °C to +27 °C. The coating was separated from the leaf surface with a soft brush, and did not undergo any further treatment to avoid solid phase reactions. The analyses were performed within a week from the collection of samples.

Isotope ratio mass spectrometry (IRMS) analysis of 13C and 18O was done on a GVI Isoprime dual inlet stable isotope mass spectrometer (GV Instruments, Manchester, UK) interfaced with a Multiprep sample preparation system. Measurements were calibrated against Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) reference standards (IAEA, Vienna, Austria). Raw δ values for oxygen were further adjusted for carbonate acid fractionation factors and converted to the VPDB scale as described previously [2].

Powder X-ray analysis was performed on a PW1710 diffractometer (Philips Analytical, Almelo, The Netherlands); scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analyses were performed on a JED-2300 energy dispersive X-ray analyzer (JEOL, Tokyo, Japan). Thermal analyses were done on an SDT 2960 thermogravimetric analyzer (TA Instruments, New Castle, DE, USA), under nitrogen gas and with a heating rate of 10 °C min⁻¹.

3 Results

The phase composition of the D. indicum mineral coating was determined from two different samples by X-ray diffraction (XRD) analysis. The first XRD test (not shown) revealed six inorganic phases in the coating: calcite (CaCO₃ trigonal), 45 wt.%; aragonite (CaCO₃ orthorhombic), 2 wt.%; monohydrocalcite (CaCO₃ · H₂O), 12 wt.%; dolomite [CaMg(CO₃)₂], 3 wt.%; dolomite [CaMg((HCO₃)OH)_2H₂O, according to [3]], 38 wt.%; and traces of silica.
To rectify these results, we repeated the analysis with Rietveld refinement, as implemented in the FullProf program [4]. The best fit to the experimental data (Figure 2) was reached when the phases of monohydrocalcite, nesquehonite, and calcite were combined with lansfordite (MgCO$_3$·5H$_2$O) and hydromagnesite [Mg$_5$(CO$_3$)$_4$(OH)$_2$·4H$_2$O]. However, the inclusion of dolomite or aragonite did not improve the refinement, so the presence of these minerals in the coating remains to be confirmed.

We performed IRMS analysis for $^{13}$C/$^{12}$C and $^{18}$O/$^{16}$O isotope ratios in the mineral phase collected from two plant specimens, and compared the results with those obtained for wadi water evaporite, surrounding mineral dust and calcareous rocks, as well as with literature data on atmospheric CO$_2$ (Table 1). The concentration of either $^{13}$C or $^{18}$O in the coating was higher than in any possible environmental carbonate source, which suggested the involvement of the plant in the formation of the mineral.

To estimate the lifetime of the mineral coating on a living plant, the following experiment was performed. Two neighboring $D.~indicum$ plants were selected. On both plants, the coating was carefully removed from a few leaves using water and brush. Simultaneously, on a few other leaves, the existing coating was marked with a food colorant. The site was then visited regularly, over the course of 10 days, to check the state of the leaves. By the end of the experiment, we observed neither the formation of new mineral nor the disappearance of the marked mineral. Therefore, the residence time of the mineral on the plant must be more than 10 days, and the week elapsing between sample collection and XRD analysis should have not influenced its composition significantly. It has been reported previously that the XRD analysis of lansfordite could be successfully completed only with a fresh sample of the mineral [5], as it readily dehydrates into an X-ray amorphous substance. Consequently, despite an improvement in XRD refinement upon the inclusion of lansfordite phase, a prolonged residence of the coating on the plant made the presence of this mineral unlikely.

The EDS analysis of the coating (Supplement A) confirmed the presence of the elements from the phases identified by XRD analysis, i.e., Ca, Mg, C, and O. In some cases, such as in plates e–h of Suppl. A, the spectrum also exhibited peaks of K together with Cl, indicating the presence of KCl, most likely in the form of sylvite mineral. The EDS image on plate g of Suppl. A showed silica traces, which were also detected in the initial XRD test. The samples of coating were not treated chemically before analysis, so the possibility of contamination could be excluded. There is at least one previous report on sylvite and silica as products of plant biomineralization in Tradescantia pallida [6].

Based on the critical analysis of XRD and EDS data, the composition of $D.~indicum$ mineral coating can be best described as having three major mineral phases: monohydrocalcite (∼13 wt.%), nesquehonite (∼38 wt.%), and calcite (∼45 wt.%); a minor hydromagnesite phase; and traces of silica and sylvite. The presence of lansfordite, aragonite and dolomite, though possible, requires additional experimental evidence.

The plot of the TGA and DTA analyses (Figure 3) revealed three pronounced endotherms, which correspond to the dehydration of nesquehonite to magnesite (MgCO$_3$) at 125 °C, followed by decomposition of the latter at 440 °C, and the decomposition of calcite at 745 °C [7]. The DTA curve does not contain the exotherm at 510 °C, which is typical for the decomposition of hydromagnesite [7]. The absence of this peak indicates that nesquehonite

![Figure 1: $D.~indicum$: Whole plant (top) and close-up of leaves (bottom), with visible whitish mineral coating (scales are approximate).](image-url)
dehydrates directly to magnesite. A small endotherm at ca. 470 °C is close to the temperature of the decarbonization of hydromagnesite [8], and a similar endotherm at 205 °C fits best with the reported dehydration of monohydrocalcite at 171 °C–206 °C [9].

4 Discussion

A positive value of δ13C in biogenic carbonates has been reported previously, but only for a few species of benthic algae [2]. Moreover, the δ13C of the D. indicum mineral coating is unusually large by magnitude, and is comparable only to that of calcareous sediments associated with seagrasses [10]. In the latter case, δ13C enrichment of the sediments had been attributed to repetitive cycles of dissolution and re-precipitation of carbonates from brine in isolated pores. A comparably large isotope fractionation has also been observed in a chemical system when calcite precipitated concurrently with monohydrocalcite [11]. The reported δ13C and δ18O values for monohydrocalcite and calcite in decaying saguaro [5] are much lower than in D. indicum coating.

The composition of the D. indicum coating bears a number of typical features of biomineralization as well as some uncommon ones. Almost the same combination of minerals has been observed in speleothems, i.e., cave mineral deposits [12, 13]. The microscopic morphology of the coating is also somewhat similar to that of the described speleothem formations in that it consists of ‘knobs’ formed by microcrystalline concentrical layers of carbonates (Figure 4).
The major component of the coating, calcite, is a frequent biogenic mineral in animals, but is seldom seen in plant tissues. The remarkable, albeit sparse, examples of higher plant calcification are cystoliths in the leaves of some plant families [14, 15], rhizoliths, i.e. formations of calcareous cementation on the surface of roots [16, 17]. There are also reports for Cactaceae as well as for some algae, fungi, and bryophytes [18, 19].

Aragonite is a metastable phase of calcium carbonate, but its transformation to calcite is negligibly slow and may take up to thousands of years [20] under normal conditions. Therefore, it cannot be considered as a transient mineral on a pathway leading to calcite, and if any aragonite had been formed by the plant, it should have remained as such in the coating. There are at least two chemical mechanisms facilitating aragonite crystallization from complex carbonate mixtures: either through partial monohydrocalcite dehydration in a wet atmosphere [21], or in the presence of elevated concentrations of Mg$^{2+}$ [22]. The nesquehonite content of the coating indicates significant amounts of magnesium in the plant exudates; however, calcium carbonate is presumably present as calcite rather than aragonite. The absence of aragonite from the coating suggests that there are no conditions favoring its formation during plant mineralization.

The magnesium carbonates nesquehonite and lansfordite (MgCO$_3$·5H$_2$O) have previously been found in decomposing saguaro [5], but neither of them is formed in the living plant. In decaying saguaro, nesquehonite was the most abundant mineral. Similarly, in D. indicum, the nesquehonite content (38%) is high but not as high as that of calcite (45%). To our knowledge, there are no other published cases of magnesium carbonates formed by plants. These minerals form in the presence of hydrocarbonate anions and magnesium cations, producing primarily lansfordite at below +4 °C, or nesquehonite at higher temperatures [5, 23]. Hydromagnesite [Mg$_5$CO$_3$$_4$·(OH)$_2$·4H$_2$O] can precipitate from solution at either high temperatures (above 52 °C) or at lower temperatures in the presence of bacteria [13, 24–27]. It is also a metastable phase, which can be transformed into more stable dolomite in the presence of Ca$^{2+}$ [27]. The average daily temperatures at the time of the formation of the mineral samples studied in the present work (15 °C – 27 °C) were far below 52 °C required for the purely chemical precipitation of hydromagnesite. This points to the involvement of the plant in this process. The absence of dolomite and aragonite from the samples, together with the major presence of nesquehonite and calcite, allows us to hypothesize that the plant exudate changes its composition periodically. It might contain high concentrations of either Ca$^{2+}$ or Mg$^{2+}$, resulting in the formation of nesquehonite and calcite, respectively. The absorption of Mg$^{2+}$ to form nesquehonite is balanced by the release of Ca$^{2+}$, which is then available for calcite formation.

Figure 3: Thermogravimetric (TG, solid line) and differential thermal analysis (DTA, dashed line) curves of D. indicum mineral coating.
but not both simultaneously. An indirect indicator of such periodical changes is the layered structure of the coating (Figure 4).

Another component of the coating, monohydrocalcite, is a common satellite mineral of nesquehonite [21]. This couple of minerals has also been found in rotten saguaro [5] and in limestone caves [13].

An uncommon feature of the coating is that it does not contain oxalates, the widespread products of plant biomineralization. Specifically, oxalate crystals are often observed in desert plants [5, 18, 28], and in association with calcite cystoliths [15]. A possible explanation could be that leaf surface-associated microorganisms participate in mineral formation. The oxalate anion could either be degraded by these microorganisms into the carbonate, or not formed at all. There are numerous reports on the complex production of dolomite, monohydrocalcite, calcite, and aragonite in the presence of microorganisms [26, 27, 29, 30]. Regarding this, in some of the SEM images (plates c, g in Suppl. A) we have observed filamentous fragments, similar to those of cyanobacteria or fungal hyphae, but their actual origin has not been determined.

Based on our data and those that have been previously published, we suggest the following scheme for D. indicum biomineralization (Figure 5). First, the plant produces an exudate with elevated concentrations of calcium or magnesium, and hydrocarbonate. If the major cation is Ca²⁺, monohydrocalcite precipitates first, according to the mechanism suggested by Fischbeck and Müller [13], which leads to an increased Mg²⁺/Ca²⁺ ratio. This ratio facilitates the precipitation of nesquehonite (or lansfordite at low temperatures) and small amounts of aragonite; moreover, Marschner reports that contact with air is essential for monohydrocalcite formation [21]. When brine contained high concentrations of Mg²⁺, the major precipitate was nesquehonite, in the same proportion as in the D. indicum mineral coating, i.e., 38%. Monohydrocalcite then gradually dehydrates either to calcite or to aragonite [31]. Aragonite forms in wet atmosphere, at temperatures ranging from −2 °C to +50 °C. In dry atmosphere, or in wet atmosphere containing ammonia, the major product of dehydration is calcite [21]. Further maturation of the mineral mixture might involve such reactions as transformation of nesquehonite to hydromagnesite [24], dissolution of nesquehonite in the exudate and subsequent crystallization of monohydrocalcite and dolomite [25], and repetitive recrystallization of the participating mineral phases leading to heavy isotope enrichment [11].

The total water content of the coating, according to the TGA analysis is 18%. This water could be important for the plant during drought periods. In addition, the coating can accumulate water both mechanically and chemically,
absorbing the morning dew and forming crystallohydrates. In either case, the formation of a set of water bearing minerals could be advantageous for a desert plant like *D. indicum*.

Overall, the discovered case of plant biomineralization is highly unusual, both with respect to the mineral phase and isotopic compositions. The mineral mixture resembles mostly the product expected from a physical crystallization of a mixed cation carbonate solution. However, its isotopic content strictly calls for the participation of the host plant in the process.

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**References**