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A spectroscopic method for identifying
terrestrial biocarbonates and application to Mars

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Abstract

Searching for traces of extinct and/or extant life on Mars is one of the major objectives for remote-sensing and *in-situ* exploration of the planet. In previous laboratory works we have investigated the infrared spectral modifications induced by thermal processing on different carbonate samples, in the form of fresh shells and fossils of different ages, whose biotic origin is easily recognisable. The goal was to discriminate them from their abiotic counterparts. In general, it is difficult to identify biotic signatures, especially when the organisms inducing the carbonate precipitation have low fossilization potential (i.e. microbes, bacteria, archaea). A wide variety of microorganisms are implicated in carbonate genesis, and their direct characterization is very difficult to evaluate by traditional methods, both in ancient sedimentary systems and even in recent environments. In the present work we apply our analysis to problematic carbonate samples, in which there is no clear evidence of controlled or induced biomineralization. This analysis indicates a very likely biotic origin of the aragonite samples under study, in agreement with the conclusion previously reported by Guido et al. (2007) who followed a completely different approach based on a complex set of sedimentary, petrographic, geochemical and biochemical analyses. We show that our method is reliable for discriminating between biotic and abiotic carbonates, and therefore it is a powerful tool in the search for life on Mars in the next generation of space missions to the planet.

Keywords: Exobiology; Mars; Spectroscopy

1. Introduction

Mars, with its arid and cold climate, is now apparently an inhospitable world for sustaining any form of life. Because of its present temperature and pressure conditions, liquid water is totally absent from the surface. However, spacecraft observations showed us a wealth of geomorphologic features that could be entirely consistent with precipitation and surface runoff (e.g. Craddock & Howard, 2002; Mangold et al., 2004; Ansan and Mangold, 2006). In fact, a warm, wet early climate capable of supporting rainfall and surface runoff is a very plausible scenario for explaining some geologic features in the Martian cratered highlands (Erkeling et al., 2010). Observations performed during several missions (both by orbiters and landers) strongly suggest that Mars was once warm and wet enough to allow the presence of abundant liquid water on the surface (e.g. Mars Pathfinder Rover team, 1997; Phillips et al., 2001; Craddock and Howard, 2002; Paige, 2005), due to a thicker atmosphere providing a strong greenhouse effect. This suggestion is supported by the results of a number of climatic models (Pollack et al., 1987; Forget and Pierrehumbert, 1997; Yung et al., 1997; Forget et al., 2004), even if this issue is not completely settled (Colaprete and Toon, 2003; Tian et al., 2010).

More recently the study of the mineralogy of Mars has independently demonstrated the role of water in the past. Mineralogical data, recovered by instruments operating in orbit and on the surface of Mars, show clear evidence for the presence of sulphates (Bibring et al., 2005; Gendrin et al., 2005), whose formation depends on the presence of liquid water (Christensen et al., 2004; Klingelhöfer et al., 2004; Squyres et al., 2004). The Mars Express spacecraft detected, together with sulfates, also phyllosilicates (clay minerals) which can be produced by the alteration of mafic or ultramafic rocks in presence of liquid water (Poulet et al., 2005; Bibring et al., 2006; Loizeau et al., 2007). The diversity of phyllosilicate mineralogy has been expanded by the observations made with the CRISM (Compact Reconnaissance Imaging Spectrometer for Mars) instrument on board of the Mars Reconnaissance Orbiter (MRO) with the identification of various hydrated silicates (Mustard et al., 2008).

New recent investigations with CRISM of the Noachian southern highlands of Mars have revealed both sulfates and phyllosilicates sometimes in intimate association (Wray et al., 2009). These assemblages are suggestive of a greater diversity of aqueous environments on ancient Mars than has previously been recognized, and their distribution implies that during the Noachian Period aqueous alteration was not restricted to a few locations but globally widespread (Wray et al., 2009).

Additional spectral and geological evidence consistent with impact-induced hydrothermal activity (Marzo et al., 2010), suggest post-Noachian formation of hydrated silicates, bringing into question models indicating that the formation of hydrated silicates is limited to the early part of the geologic record of the planet (Bibring et al., 2006).

Mars orbital spectral data have provided evidence also for carbonates (Bandfield et al., 2003; Ehlmann et al., 2008; Palomba et al., 2009; Murchie et al., 2009) while Morris et al. (2010), using the rover Spirit have recently identified outcrops rich in Mg-Fe carbonate (16 to 34 wt.%) in the Columbia Hills of Gusev crater. Interestingly, its composition approximates the average composition of the carbonate globules in Martian meteorite ALH84001. The presence of carbonates, probably precipitated from carbonate-bearing solutions, imply that neutral-to alkaline waters existed at the time of their formation.

All the above mineralogical evidence suggests that several distinct habitable niches may thus have been present on ancient Mars, providing a range of opportunities for the origin of life, including places of impact-induced hydrothermal systems (Newsom, 1980; Gulick and Baker, 1989, 1990; Gulick, 1998, Marzo et al., 2010). This hypothesis provides not only an interesting example of post-Noachian aqueous alteration on Mars, but suggests also further sites of astrobiological potential.

Searching for traces of extinct or extant life is a major objective of Mars exploration, even though the detection of a biosignature is not a straightforward process (e.g. Marzo et al., 2007). However, the probability of finding traces of biological activity would certainly be higher if the search is directed towards materials whose origin can be traced back to some form of life. This is the case with some terrestrial living organisms which are able to produce mineral matrices in the so called

biomineralization process (Lowenstam, 1981; Mann, 1983, 2001). Nevertheless the problem of discriminating between biominerals and their abiotic counterparts is far from trivial. However, by means of thermal processing, it is possible to distinguish, using differential thermal analysis (Cabane et al., 2004; Stalport et al., 2005, 2007) or infrared (IR) spectroscopy (Orofino et al., 2007), abiotic calcium carbonate minerals (CaCO_3 i.e. aragonite or calcite) from the corresponding biominerals. In a series of recent papers we have developed and applied our method (D'Elia et al., 2007; Orofino et al., 2007, 2009, 2010) to different carbonate samples in the form of fresh shells and fossils of different ages found in different places and easily recognizable as of biotic origin. In this work, applying our analysis to problematic carbonate samples, in which there is no clear evidence of controlled or induced biomineralization, we show that the method is reliable, allowing for the discrimination of biotic and abiotic carbonates.

2. Previous work and method

The polymorphs of CaCO_3 , aragonite and calcite, are interesting because on Earth these compounds are produced by abiotic processes as well as by biological activity. According to Mackenzie (1970), when aragonite (a metastable form of CaCO_3 present in almost all mollusc shells) undergoes heat treatment, an endothermic transformation into calcite (the stable state of CaCO_3) occurs. Then, after further heating, calcite decomposes into solid calcium oxide (CaO) and gaseous carbon dioxide (CO_2). The latter transformation is complete at 800 - 1000 °C (Stalport et al., 2005, 2007), but it begins at a lower temperature which depends on the biotic or abiotic origin of the sample. In the case of recent biominerals, heating at 485 °C for 3.5 hours is sufficient to start altering their chemical and/or physical properties, while for minerals of abiotic origin higher temperatures are necessary (see Orofino et al., 2007, 2009, for details). As described in previous work, all the samples were ground for approximately 10 minutes in a mechanical mortar grinder in order to obtain fine powders suitable for IR spectral analyses. The thermal processing of the samples was performed by means of a furnace under vacuum.

To study the above mentioned transformations we performed IR transmission spectroscopy in the range 2000 - 370 cm^{-1} , focusing our attention only on the last part of the spectra (700 - 370 cm^{-1}). This allows to monitor the decomposition process undergone by our samples giving, at the same time, the advantage of using only one type of matrix (potassium bromide, KBr) for the IR transmission spectroscopy in the range 2000 - 370 cm^{-1} (Orofino et al. 2009). For all the samples we measured the spectra before and after the heating treatment at 485 °C for 3.5 hours. In Fig. 1 we compare, as an example, the spectra obtained before and after such a treatment for a fresh shell (*Tellina exigua*).

Figure 1

Analysing the solid curve in Fig. 1, it is possible to infer that the sample is composed of aragonite, due to the presence of the relevant features at 1478 cm^{-1} (6.8 μm), 1083 cm^{-1} (9.2 μm), 859 cm^{-1} (11.6 μm) and a double peak around 700 cm^{-1} (14.3 μm), characteristic of this mineral (White, 1974). The dotted line shows the spectrum obtained for the sample thermally processed at 485 °C. The transformation from the metastable form of aragonite to the more stable form of calcite is indicated by the loss of the feature at 1083 cm^{-1} and of the doublet at 700 cm^{-1} , where we observe, instead, a single transmittance minimum. In addition, the ongoing decomposition of CaCO_3 into CaO (and CO_2) starts to appear, since the bands at 1083 and 859 cm^{-1} become weaker and the transmittance below 700 cm^{-1} starts to decrease considerably due to the onset of the broad band centered at 330 cm^{-1} (30.3 μm) characteristic of CaO (Farmer, 1974), partially shown in Fig. 1. For comparison in Fig. 2 we report the transmission spectrum of a sample of CaO grains (supplied by Sigma–Aldrich Co.) obtained in our laboratory. In the same figure are reported the reference spectra of particulate samples of calcite and aragonite obtained by Salisbury et al. (1991).

Figure 2

For every spectrum of each processed and unprocessed sample, we have evaluated the transmittance ratios, R_p and R_u respectively:

$$R_p = T_p(v_2) / T_p(v_1)$$

$$R_u = T_u(v_2) / T_u(v_1)$$

where $v_1 = 650 \text{ cm}^{-1}$ ($15.4 \text{ }\mu\text{m}$) and $v_2 = 510 \text{ cm}^{-1}$ ($19.6 \text{ }\mu\text{m}$) are two wavenumbers chosen in the spectral range of interest where the CaO band begins to develop (the subscripts p and u refer to processed and unprocessed samples respectively).

Taking into account all the spectra relative to a given sample, we have averaged the values of R_u and R_p and, from these two mean ratios $\langle R_u \rangle$ and $\langle R_p \rangle$, we have obtained for each sample a slope variation index:

$$D = \langle R_p \rangle / \langle R_u \rangle.$$

The index D estimates the slope variation of the spectra obtained after heating at $485 \text{ }^\circ\text{C}$ with respect to that measured for the unprocessed sample. Due to the fact that the spectral slope is always steeper in the case of the processed sample, $D \leq 1$ for all the samples. We note that as D increases, the spectral slope of the processed sample becomes more similar to that of the unprocessed one, meaning that the thermal processing is less effective in the transformation of CaCO_3 into CaO.

Taking into account the samples described in Orofino et al. (2010), i.e. recent shells (*Tellina exigua*, *Cardites antiquata*), fossils of various geologic time from the Holocene to the Mesoproterozoic Era (*Pecten* sp., *Ostrea* sp., *Myriapora truncata*, *Clypeaster* sp., *Haliotis lamellosa*, rudist bivalves, *Ortocheras* sp., fossil stromatolites) and aragonite and calcite minerals, we found a correlation between the age of a biotic carbonate sample and the relative spectral slope, in the range $700 - 370 \text{ cm}^{-1}$, of the heated sample with respect to that of the unprocessed one.

The D index is plotted in Fig. 3 versus the logarithm of the stratigraphic age of each sample. The beginning and the end of each period/epoch are those established by the International Commission on Stratigraphy (ICS) devoted to the terrestrial stratigraphy on a global scale (Gradstein et al., 2004;

Ogg et al., 2008). In this respect we have chosen the central age of the period the sample dates from, with an uncertainty equal to $\pm \Delta/2$, where Δ is the duration of each period. As far as the error on the D index is concerned, all the vertical error bars reported in Fig. 3 refer to the larger uncertainty between the statistical dispersion, derived from the error analysis of the various measurements performed on the same sample, and the instrumental accuracy of the transmittance spectra (typically 1%).

Figure 3

The linear trend shown in Fig. 3 is reasonably well fitted by the function:

$$D = a \log t + b$$

where t is the age of the sample (in Myr), $a = 0.0125$ and $b = 0.8666$; we find a correlation coefficient $r \cong 0.95$ (Taylor, 1982).

This correlation implies that the recent fossils maintain the characteristic “fragility” of fresh biominerals – see Orofino et al. (2007) for details – while for more ancient fossils the spectroscopic behavior, after heat treatment, becomes increasingly similar to that of abiotic minerals and the D index gets closer to 1. Therefore D could be seen as an index of degradation of the fossil, in the sense that $D = 1$ implies the impossibility of discriminating between biotic and abiotic carbonate samples. The straight line represents therefore the place where the D index values of “normal” biotic carbonates fall as a function of the age, at least up to about 1 Gyr in terrestrial environments.

A deviation from this linear trend may indicate a particular situation and/or a different evolution of the biotic carbonate sample under analysis. In this context, we recently found that a group of terrestrial fossils embedded in a layer of clays exhibit values of D smaller than their coeval fossils found in other mineralogical environments. We interpreted such behavior as the result of a favourable condition for preserving their biomineral characteristics (Orofino et al., 2010). An

interesting consequence of this result is that the phyllosilicates regions recently discovered on Mars may represent very interesting environments that can provide conditions favourable to preserve evidence of biomarkers. Therefore they can be regarded as good candidate locations for biosignature detection.

As far as very old biotic samples are concerned we have found that on Earth a billion years is long enough to produce a complete degradation of the fossil structure, giving rise to post-processed spectral behaviors that are very similar to those of unprocessed samples ($D \cong 1$).

It is worthwhile to stress the importance of the heating of the samples as a powerful means to evaluate their level of degradation, since samples which experienced different degrees of degradation react in different way to thermal processing probably due to the different arrangement of atoms into the structure of the fossil. The IR spectroscopy of the sample after heating at 485 °C allows discriminating its origin, by means of the evaluation of the D parameter, as previously described.

3. The new carbonate rock samples

Mineral deposits resulting from microbially-induced mineralization are called microbialites (Burne and Moore, 1987). Other terms such as microbolite (Riding, 1991), automicrite (e.g. Wolf, 1965; Reitner et al., 1995) or organomicrite (Reitner et al., 1995) are also used. Most microbialites can be classified into one of three main categories based on their macroscopic features (Riding, 1991; Dupraz and Strasser, 1999): stromatolites, showing a laminated macrofabric (Monty, 1977; Semikhatov et al., 1979), thrombolites, displaying a clotted (mesoclots) macrofabric (Aitken, 1967; Kennard and James, 1986; Turner et al., 2000; Shapiro, 2000), and leiolites, without well defined macrofabric (Braga et al., 1995). These three types of microbialites can display a wide range of microstructures including micropeloidal, densely micritic, or agglutinated microfabrics (Riding, 1991; Dupraz and Strasser, 1999). One of the major problems is the characterization of the micrite component. The term micrite, introduced by Folk (1959), is the abbreviation of “microcrystalline

calcite” and is commonly used to describe very fine carbonate particles less than 4 microns in size. Particularly important is the distinction between biotic and abiotic micrite. Micromorphological observations can furnish an initial contribution to understand the origin of the micrites but in some cases they do not show any diagnostic features even if they are biotic in origin. For this reason the recognition of microbial activity is very difficult in ancient carbonates.

As already mentioned in Section 1, in this work we present the analysis and the measurements performed with our method on some carbonate samples in which there are no macroscopic evidences of controlled or induced biomineralization.

The carbonate samples used in our study come from the Calcare di Base (CB) formation, which crops out in the Rossano Basin (Northern Calabria, Italy) and formed in the Late Miocene (Messinian, ~ 6 Myr – see below). In the study area the CB succession consists of two massive fine-grained calcareous beds interlayered with decimetric laminated marls. In such geological settings the CB formation is massive, without any evidence of evaporitic minerals, and is preserved as aragonite (Guido et al., 2007; Rao et al., 2006).

3.1 Origin and properties

The six bulk CB samples, labelled CB1-CB6, were collected near the village of Cropalati and selected according to their position in the stratigraphic succession (see panel A in Fig. 4) and to the lithological features observed in the field (see panel B in Fig. 4). These samples were previously studied and characterized through molecular fossils and other organic markers (Guido et al., 2007).

Figure 4

The origin of these deposits (biotic *versus* abiotic) is not clear, due to the absence of fossils and well-defined sedimentary structures. While several authors interpreted their genesis as evaporitic and/or diagenetic (Rouchy and Caruso, 2006), recently Guido et al. (2007) demonstrated that the CB carbonates are preserved in their original aragonitic mineralogical phase and are composed of

small crystals organized as peloids forming clots with an antigravitative fabric (i. e. an arrangement that is not grain supported and with large interclot areas showing a fabric contrary to what dictated by gravity) The bright UV fluorescence indicates that peloidal micrite is rich in organic matter. Guido et al. (2007), using an organic matter study performed through Rock-Eval pyrolysis analyses, palynofacies observations and molecular-fossil recognition, demonstrated that carbonate precipitation was induced by bacterial ammonification of amino acids in aerobic conditions. Therefore these deposits are the results of biotic mineralization in a marine depositional scenario influenced by freshwater input rather than evaporitic concentration. These problematic carbonates seem to represent a good sample to validate the applicability of the IR transmission spectroscopy in the discrimination between biotic and abiotic carbonates.

3.2 Analysis and measurements

The samples were ground to obtain fine powders and analysed by means of IR transmission spectroscopy before and after thermal processing at 485 °C for 3.5 hours. All of them exhibit the same trend and, as examples, we show in Figs. 5a and 5b the spectra of samples CB1 and CB4 (see panel A in Fig. 4 for their location). As it can be clearly seen the transmission spectra before thermal processing (solid lines in Figs. 5a and 5b) exhibit the typical absorption bands of aragonite at 1476 cm⁻¹ (6.8 µm), 1083 cm⁻¹ (9.2 µm), 856 cm⁻¹ (11.7 µm) and a double peak around 700 cm⁻¹ (14.3 µm), characteristic of this mineral (White, 1974). The position of the main bands in the spectra after thermal processing reveals that the transformation to calcite has occurred while the changes in the slope beyond 700 cm⁻¹ indicates that the conversion to CaO also began (for the details see Orofino et al., 2007, 2009). The presence in the spectra, both before and after thermal processing, of other features in the 1200 – 1000 cm⁻¹ range and the doublet around 630 cm⁻¹, indicates the existence of some sulphate, most probably celestine (SrSO₄) as suggested also by our laboratory EDX (Energy Dispersive X-ray) analysis of the samples.

Figure 5a and 5b

The evaluation of the slope variation index D for the six samples and the knowledge of their geologic age, allow their positioning in the plot of Fig. 3, where we report the average value of this parameter along with its error bar, due to uncertainty on the single samples.

The age of the samples (~ 6 Myr) has been inferred from their stratigraphic position while its uncertainty, as usual, has been assumed equal to half of the duration of the Messinian period, according to Gradstein et al. (2004)

As it can be seen in Fig. 3, the point representing our samples, in what we can define as the “biotic carbonate diagram”, clearly indicates a very likely biotic origin of the aragonite under analysis. This conclusion is in good agreement with that obtained by Guido et al. (2007) using a completely different approach based on complex geochemical techniques.

4. Discussion and conclusions

In the framework of the search for life on Mars, a method able to discriminate between biotic and abiotic origin of carbonate samples is certainly of valuable importance for the next generation of space missions to the planet. In previous work (D’Elia et al., 2007; Orofino et al., 2007, 2009, 2010) we have developed a method to distinguish, by means of IR spectroscopy, abiotic calcium carbonate minerals from the corresponding biominerals in form of fresh shells and fossils found in different places and easily recognizable as of biotic origin. The IR spectroscopy of the sample after heating at 485°C allows discriminating its origin, by means of the evaluation of the D parameter (see Section 2).

The linear correlation found between the D index and the logarithm of the age of known biotic samples, which we named “biotic carbonate diagram”, can be used to:

- a) discriminate between biotic and abiotic origin of other carbonate samples;
- b) individuate particular groups of carbonate fossils;
- c) estimate the age of terrestrial samples of known biotic origin.

The evaluation of the age of the samples (point (c)) can be easily understood and applied. Concerning point (b), the deviation from the linear trend has been already evidenced for a group of terrestrial fossils embedded in a layer of clays, with important implications in the search for biosignatures on Mars (Orofino et al., 2010).

As far as point (a) is concerned, in this work we have applied our analysis to carbonate samples, in which there is no clear evidence of controlled or induced biomineralization, and we have shown that the method is reliable and useful to discriminate between biotic and abiotic carbonates, even in the absence of fossils and well-defined sedimentary structures. Indeed, as it can be seen from Fig. 3, the mean position of these samples in the “biotic carbonate diagram”, clearly indicates a very likely biotic origin of the aragonite samples under analysis. This is in agreement with the conclusion reported by Guido et al. (2007) who followed a completely different approach based on a complex set of sedimentary, petrographic, geochemical and biochemical analyses.

It is worthwhile to note the relative simplicity of our method compared to the sophisticated geobiological investigations already applied and discussed by Guido et al. (2007). On the other hand, we acknowledge a possible drawback of our method due to the relatively small number of samples used to validate it. For this reason future work will be devoted to increase the number of analysed samples. In addition, it is necessary to recall that our samples are, on the geological scale, relatively young (less than 1 Gyr old), while the hypothetical Martian fossils are expected to be older. In fact it is likely that the initial conditions favorable to life, with a thick atmosphere able to allow abundant liquid water on the surface of the planet, lasted only 1 Gyr or less (Pollack et al., 1987), so it is possible that we would not find fossils, if any, younger than 3.5 Gyr old. These fossils could be so degraded that they would be indistinguishable from abiotic carbonates.

It is important to note, however, that in the last 3.5 Gyr the geologic and climate conditions on Mars have been quite different from those present on Earth. In particular, while in the terrestrial environment the circulation of water in the ground has chemically altered the nature of biotic materials, the likely absence of such conditions on Mars for most of its history may have preserved

some biotic signatures even for samples as old as 3.5 Gyr. Such possibility is also reported and discussed by Stalport et al. (2005), leading to the same conclusions. Due to the very different evolution of the environmental conditions on Mars and Earth, it is not unreasonable to think that the ancient Martian biotic carbonates, if they exist, may have experienced a degradation process much slower than that on Earth and therefore they could have a degradation level comparable to that of much younger terrestrial analogues. If so, such Martian biotic carbonates could be detected by means of IR spectral analyses, making the “biotic carbonate diagram” a powerful tool for the search for life on Mars. This means that experimental projects designed as *in-situ* or sample-return analyses and based on IR spectroscopy, could be useful as diagnostic technique complementary and/or alternative to the DTA analysis already proposed for the same purpose (Cabane et al., 2004; Stalport et al., 2005, 2007). The choice of the more suitable procedure may obviously be dictated by several considerations and opportunities which at the moment are beyond the aim of the present work.

As mentioned before, in the future we plan to increase the number of analysed fossils in order to improve the statistical significance of the “biotic carbonate diagram”. The analysis of fossils older than those studied up to now, will be also useful in order to investigate the spectral reaction to heat processing of less preserved biological structures. Furthermore, as already pointed out, it will be possible to apply our method to discriminate between biotic and abiotic origin of other carbonate samples coming from different localities (e.g. Brown et al., 2010).

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Figure captions

Figure 1. IR spectra of *Tellina exigua* fresh shell obtained before (solid line) and after (dotted line) thermal processing for 3.5 hours at 485 °C.

Figure 2. Reference spectra of aragonite and calcite (data from Salisbury et al., 1991) and pure CaO grains analyzed in our laboratory. The spectra have been arbitrarily scaled for sake of clarity.

Figure 3. Values of the slope variation index D , calculated for all the samples (from #1 to #12) analysed by Orofino et al. (2010) and plotted versus the age of the samples. The D values determined for minerals of abiotic origin (samples #11 and #12) have to be considered outside the chronological range. The arrow indicates the average value (#13) for the CB samples studied in the present work (see text, Sect. 3).

Figure 4. Study section (panel A) and general view of the Rossano Basin (panel B) showing the position where the six samples of Calcare di Base have been collected. The black box in B indicates the location shown in A.

Figure 5a. IR transmission spectra of sample CB1 before (solid curve) and after (dotted curve) thermal processing for 3.5 hours at 485 °C.

Figure 5b. Same as Fig. 5a, but for sample CB4.

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617 > We present a spectroscopic method to discriminate biotic from abiotic carbonates. > Infrared
618 spectral modifications induced by thermal processing are investigated. > We analyse carbonate
619 samples with no evidences of biomineralization. > Our method is a powerful tool for the search of
620 life on Mars.

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