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FT-Raman microscopic studies of *Haematomma ochroleucum* var. *porphyrium*

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Abstract: The FT-Raman spectra of thallus-substratum encrustations of the lichen *Haematomma ochroleucum* var. *porphyrium* on 18th century brickwork have been obtained successfully, despite the acknowledged fragility of this species. Spectra from the upper and lower surfaces of the encrustations are different; the spectra from the upper surface contain vibrational features which are assignable to the lichen, whereas those from the lower surface are dominated by bands arising from calcium oxalate and oxalic acid. Other features present in the vibrational spectra are ascribed to phenolic compounds from lichen metabolism.

Introduction

Most physical methods of microanalysis are based on atomic properties and thus only indirectly provide information on chemically-bonded structures. FT-Raman microscopy is a new technique for microanalytical structural investigation. An advantage of this type of laser microscopy, in addition to the very small amounts of material required for analysis, is the low laser power needed for sample illumination; non-destructive analysis is thereby achieved and this can be important for sensitive, valuable samples. In this paper a novel application of FT-Raman microscopy to the identification of chemical compounds involved in the biodeterioration of brickwork is described.

Previous Raman spectroscopic studies from our laboratories (EDWARDS *et al.* 1991, 1991b, 1992) have demonstrated the capability of the technique for the investigation of the chemical composition of lichen-substratum interfaces. Using conventional and FT-Raman spectroscopic methods, with visible and

near infrared laser excitation, quality spectra have been obtained from macroscopic and microscopic samples in the form of scalpel-excised and *in situ* encrustations (EDWARDS & SEAWARD 1993). The identification of calcium oxalate monohydrate as a major component (~ 45%) of the encrustations of *Dirina massiliensis* f. *sorediata* and *Ochrolechia parella* thalli on man-made and natural substrata has been contrasted (EDWARDS *et al.* 1994) with *in situ* measurements of *Xanthoria parietina* on sandstone, which contains only small amounts of calcium oxalate monohydrate (~ 2%).

In addition, the presence of incorporated substrata material such as calcite, gypsum and paint pigments into the encrustations formed by the action of *Dirina massiliensis* f. *sorediata* on Renaissance frescoes has been shown (EDWARDS *et al.* 1991b); the encrustations have been described as complex, heterogeneous sites of chemical activity (JONES & WILSON 1985; SEAWARD & GIACOBINI 1988). In the case of *Xanthoria parietina*, the bright orange pigment of the living species provided an additional problem because of the fluorescence background with the visible laser excitation wavelengths in the range 488-515 nm. However, the advantage of near infrared excitation at 1064 nm was immediately apparent in this particular case (EDWARDS *et al.* 1994).

In all our previous studies involving excised samples, the lichen thallus material was coherently detached from the substrata, even in the case of the aggressive thalli of *Dirina massiliensis* f. *sorediata* for which the relatively large and friable encrustations required very careful physical handling. In the present study, we report the results of a Raman spectroscopic study of excised samples of *Haematomma ochroleucum* (Neck.) J.R.Laundon var. *porphyrium* (Pers.) J.R.Laundon encrustations from a brick substratum. This lichen is noted for its friability and intolerance to handling; hence, the power and versatility of the Raman spectroscopic technique in the obtaining of spectra non-destructively from such delicate specimens would be a novel application in this field of study.

There is no previous vibrational spectroscopic work on this species, but recent chemical analyses (LMS & TLC) undertaken for us by Prof. C. LEUCKERT and Dr H. KÜMMERLING (Freie Universität Berlin) have shown the presence of atranorin, usnic acid (trace), bourgeanic acid, zeorin and traces of unidentified unknowns - apparently a new chemotype according to Mr J. R. LAUNDON (*in litt.*).

Experimental

Several samples of *H. ochroleucum* var. *porphyrium* were obtained from early 18th century brick walls surrounding Goxhill Hall, Lincolnshire (grid ref. 54/110.206); the samples were taken from regions which were not overlapping with the mortar cement and excised with care, since this lichen-substratum system is very friable.

FT-Raman microscope spectra were excited using a Nd:YAG laser operating at 1064 nm with powers of about 20-100 mW and a Bruker IFS 66 far-infrared

instrument with FRA 106 Raman module attachment and Ramanscope (EDWARDS & SEAWARD 1993). Microscope objectives of 20x and 40x were used in this study, giving spot sizes of about 10-20 μm at the focus. Overall magnifications were of the order 2000x. Low laser powers were used for most of the spectra because of the observation of sample damage with laser powers greater than about 200-350 mW. With power levels of around 50 mW or so, there was no observable evidence of sample degradation even after extended data accumulation of 24,000 scans (representing about 12 h continuous illumination by the laser beam). Normally, about 2000-12,000 accumulated scans were required to achieve satisfactory signal-to-noise ratios for spectroscopic analysis.

Spectroscopic data were analysed from several regions of the encrustations on the upper surface of the lichen and at the lichen-substratum interface; the latter was observed to be more heterogeneous, both visually at high magnifications and spectroscopically.

Results and discussion

FT-Raman spectra obtained from the upper and lower surfaces of the encrustations are shown in Figs. 1 and 2. The wavenumbers of the spectral features and approximate assignments of the vibrational modes are given in Tab. 1; there are, of course, some differences between the spectra obtained from different sampling regions of the same surface for each encrustation, but a pattern does emerge from these spectra and the wavenumbers given in Tab. 1. The following points may be noted:

(i) The upper surface, which appears visually to be more homogeneous, has some vibrational features which may be attributed to the lichen body, such as the medium-intensity band at 1525 cm^{-1} and weaker features at 1381 , 1296 and 1157 cm^{-1} .

(ii) Spectra from the lower surface are dominated by the bands due to calcium oxalate monohydrate, which is still observed at the upper surface of the lichen-substratum, although some organic material is present due to the $\nu(\text{CH})$ stretching features in the region of 2900 cm^{-1} and $\delta(\text{CH}_2)$ bands around 1300 cm^{-1} . At the lower surface, which is thought to be a site of considerable chemical activity, the presence of lichen metabolic products would be expected, especially phenolic and ring carboxylate compounds.

(iii) Free crystalline oxalic acid is identifiable by the band at 1626 cm^{-1} at the lower surface (EDWARDS *et al.* 1991b), presumably from lichen metabolic production and which has only partially reacted with the brick substratum to produce calcium oxalate monohydrate. In previous studies of *Dirina massiliensis* f. *sorediata* encrustations on calcite or marble, there was no evidence for free oxalic acid which had reacted fully to produce calcium oxalate (EDWARDS *et al.* 1991, 1991b, 1992).

(iv) Raman bands at 1452 and 1658 cm^{-1} are noted in spectra from the upper surface regions; these are of interest since they are assignable to complexed oxalates coordinated to metal ions (BICKLEY *et al.* 1991; EDWARDS *et al.* 1991a). It is believed that this could be a mechanism for the organism dealing with heavy metal contaminants in the substratum through complex coordination and chelation (PURVIS 1984; WILSON & JONES 1984). It is not possible at the moment to identify which metals could be involved here, but magnesium or a transition metal such as iron could be considered.

(v) Other parts of the heterogeneous encrustation at the lower surface have yielded spectra which are somewhat different from those discussed above, *e. g.* Fig. 3. This spectrum is almost entirely assignable to oxalates in several chemical forms, such as oxalic acid, free oxalate ions and complex chelated metal-oxalate. Characteristic features of the latter compounds are the bands in the region 100-600 cm^{-1} which are assignable to the metal-oxygen stretching and $\delta(\text{CCO})$ ring deformation vibrations (BICKLEY *et al.* 1991; EDWARDS *et al.* 1991a).

Other particulate matter observed locally in these regions gives spectra which are exemplified by Fig. 4, which is typical of that of gypsum and is presumed to arise from mortar contamination. The origin of the strongest gypsum feature at 1009 cm^{-1} , which is observed in the spectra of the upper surfaces, is of interest since it could arise from dust contamination, wash-off from other lichen growths in the vicinity of the sampled area or alternatively, by assimilation into the body of the lichen from the encrustation.

In conclusion, a Raman spectroscopic study of the lichen-substratum system of *Haematomma ochroleucum* var. *porphyrium* growing on brickwork indicates several novel features which have hitherto not been recorded for other systems, such as those associated with *Dirina massiliensis* f. *sorediata* or *Xanthoria parietina* (EDWARDS *et al.* 1991b; EDWARDS & SEAWARD 1993; EDWARDS *et al.* 1994).

Tab. 1. Wavenumbers/ cm^{-1} and vibrational assignments for the Raman spectra of *Haematomma ochroleucum* var. *porphyrium* lichen-substratum encrustations on brickwork.

Lichen-substratum		Approximate description of vibrational mode
Upper surface v/cm^{-1}	Lower surface v/cm^{-1}	
2970 w,sh		$\text{v}(\text{CH}_3)$ asymmetric stretch
2936 m	2938 mw,br	$\text{v}(\text{CH}_2)$ symmetric stretch
2880 mw,sh	2885 w,sh	$\text{v}(\text{CH}_3)$ asymmetric stretch
1658 w		$\text{v}(\text{CO}_2)$ stretching, coordinated oxalate
	1626 m,br	$\text{v}(\text{CO}_2)$ symmetric stretching, oxalic acid
1525 ms		$\text{v}(\text{CC})$ lichen carotenoids
1475 m	1474 ms	$\text{v}(\text{CO}_2)$ symmetric stretching, calcium oxalate
1452 w,sh		$\text{v}(\text{CO}_2)$ stretching, coordinated oxalate
1381 mw,sh		$\delta(\text{CH}_2)$ cellulose
	1304 m,br	$\delta(\text{CH}_2)$
1296 m,br		$\delta(\text{CH}_2)$ cellulose
1157 m		$\text{v}(\text{CO}_2)$ cellulose
	1115 w,br	$\text{v}(\text{CO}_2)$ cellulose
1080 mw		$\text{v}(\text{CO}_3^{2-})$ symmetric stretching, calcite
1009 mw		$\text{v}(\text{SO}_4)$ symmetric stretching, calcium sulphate
910 w	908 m	$\text{v}(\text{CC})$ calcium oxalate
506 w		$\delta(\text{CO}_2)$ calcium oxalate
	355 w	$\delta(\text{COH})$ torsion, oxalic acid
	189 m	

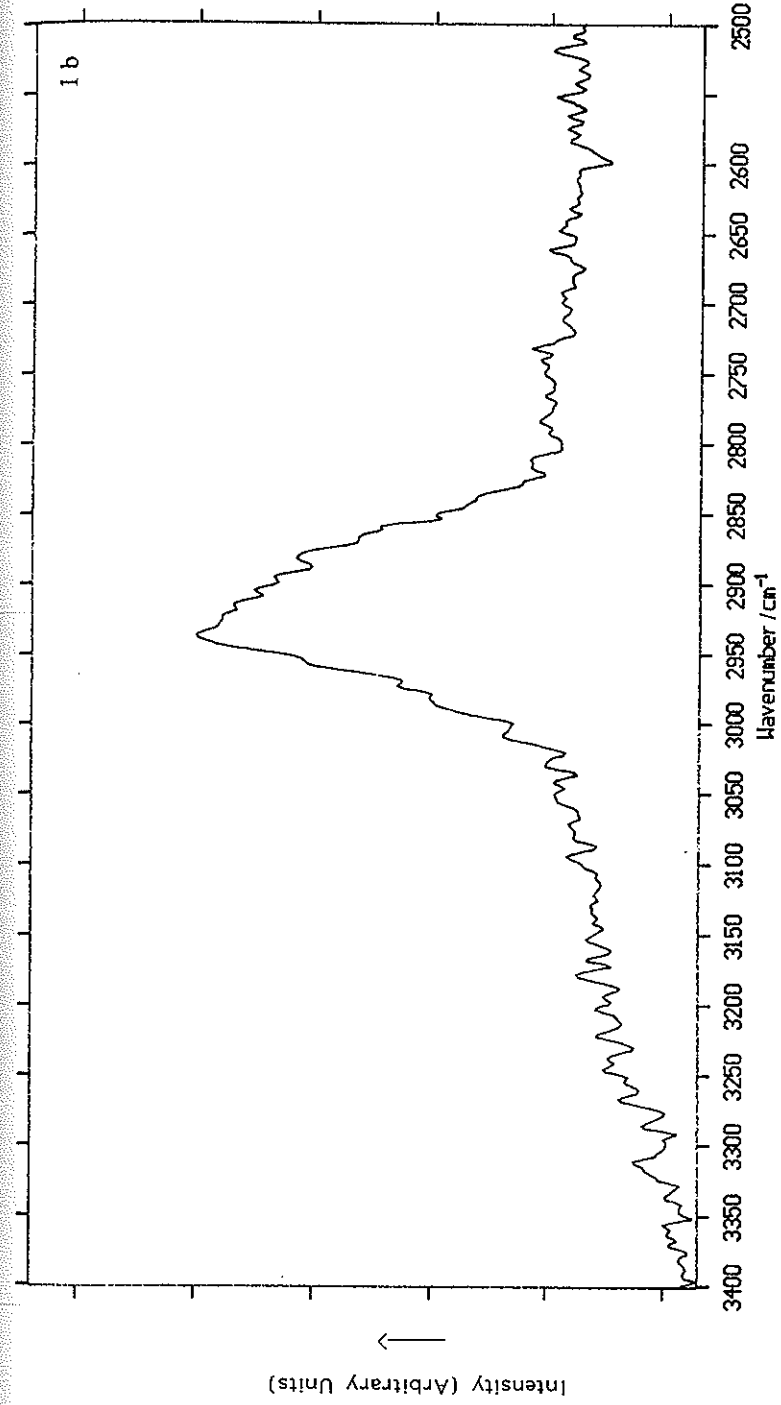
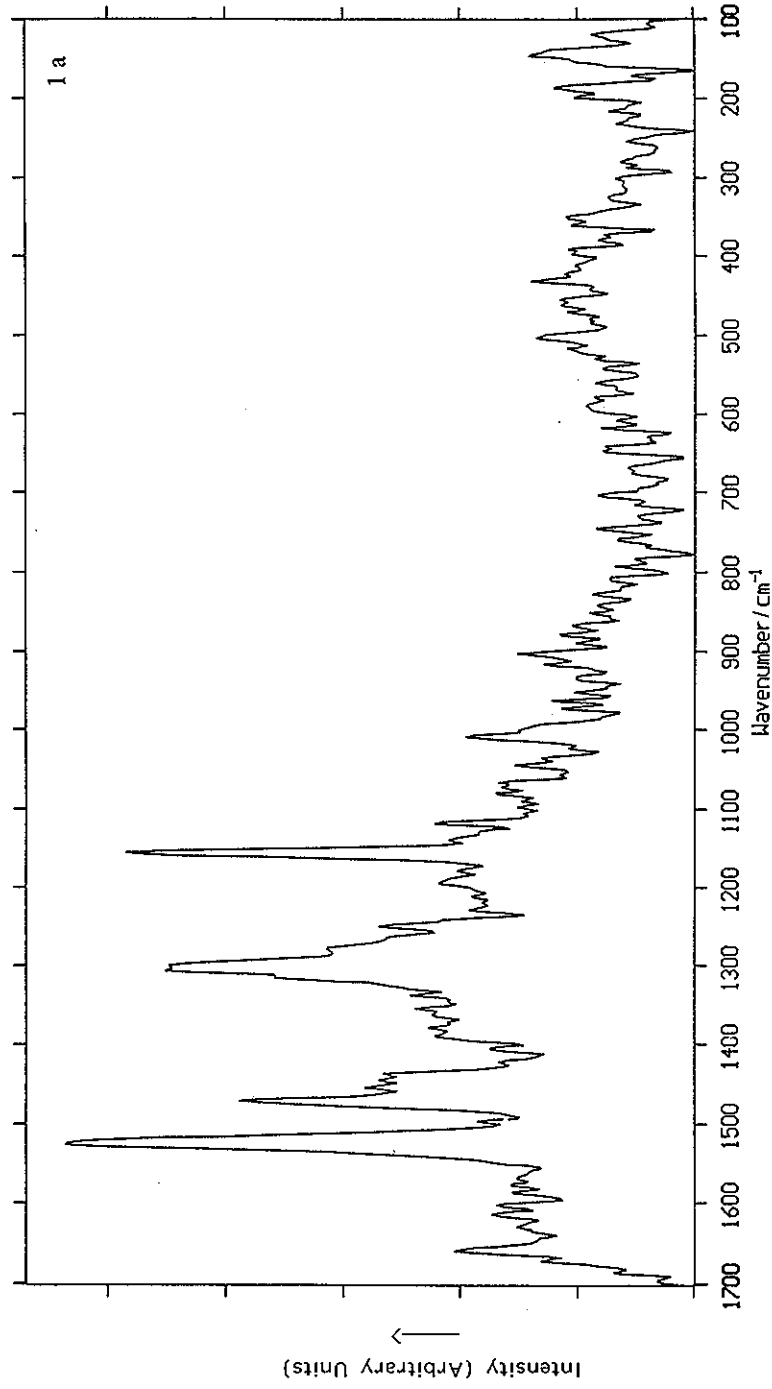


Fig. 1. Raman spectrum of *Haematoma ochroleucum* var. *porphyrium* encrustation, upper surface, 1064 nm excitation; 40x microscope objective (a) 100-1700 cm^{-1} wavenumber range (b) 2500-3400 cm^{-1} wavenumber range; resolution 4 cm^{-1} ; 12,000 scans.

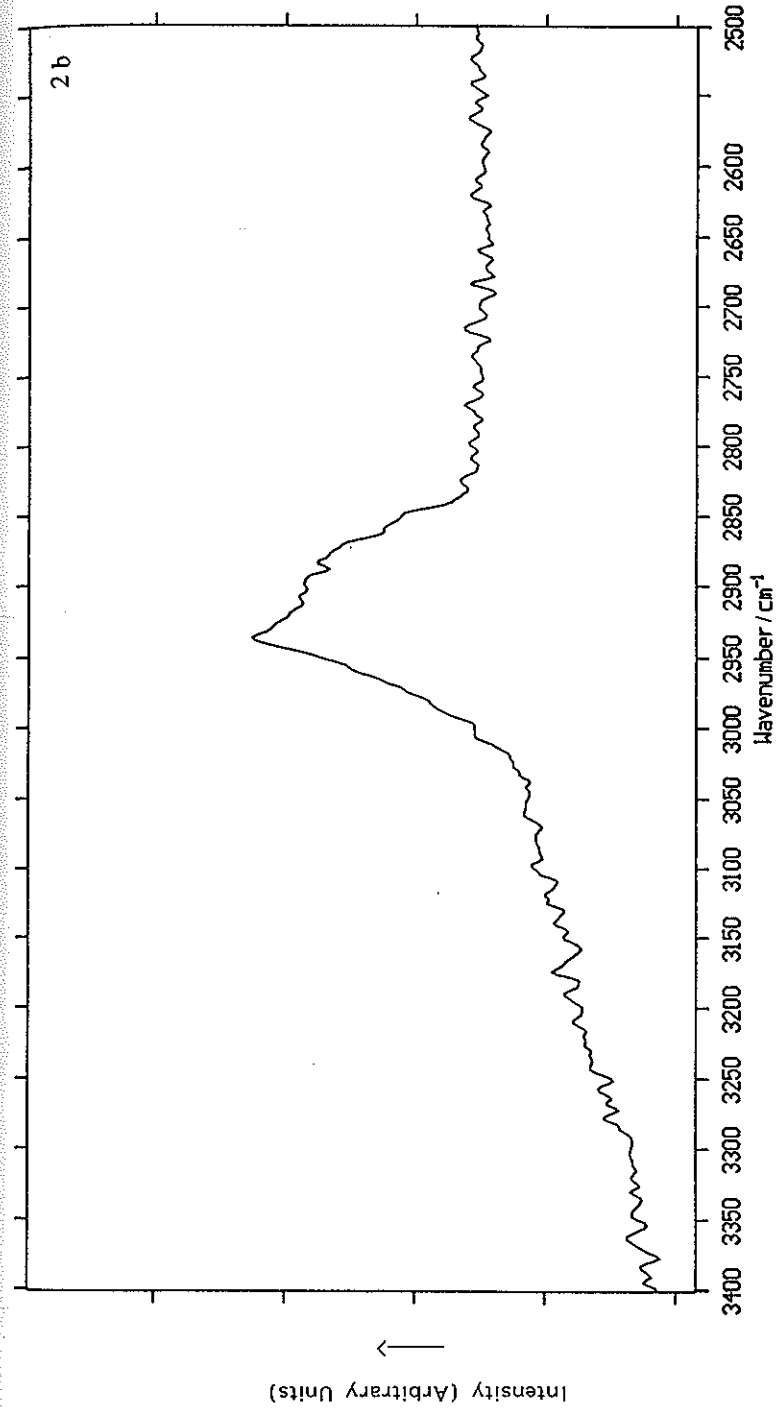
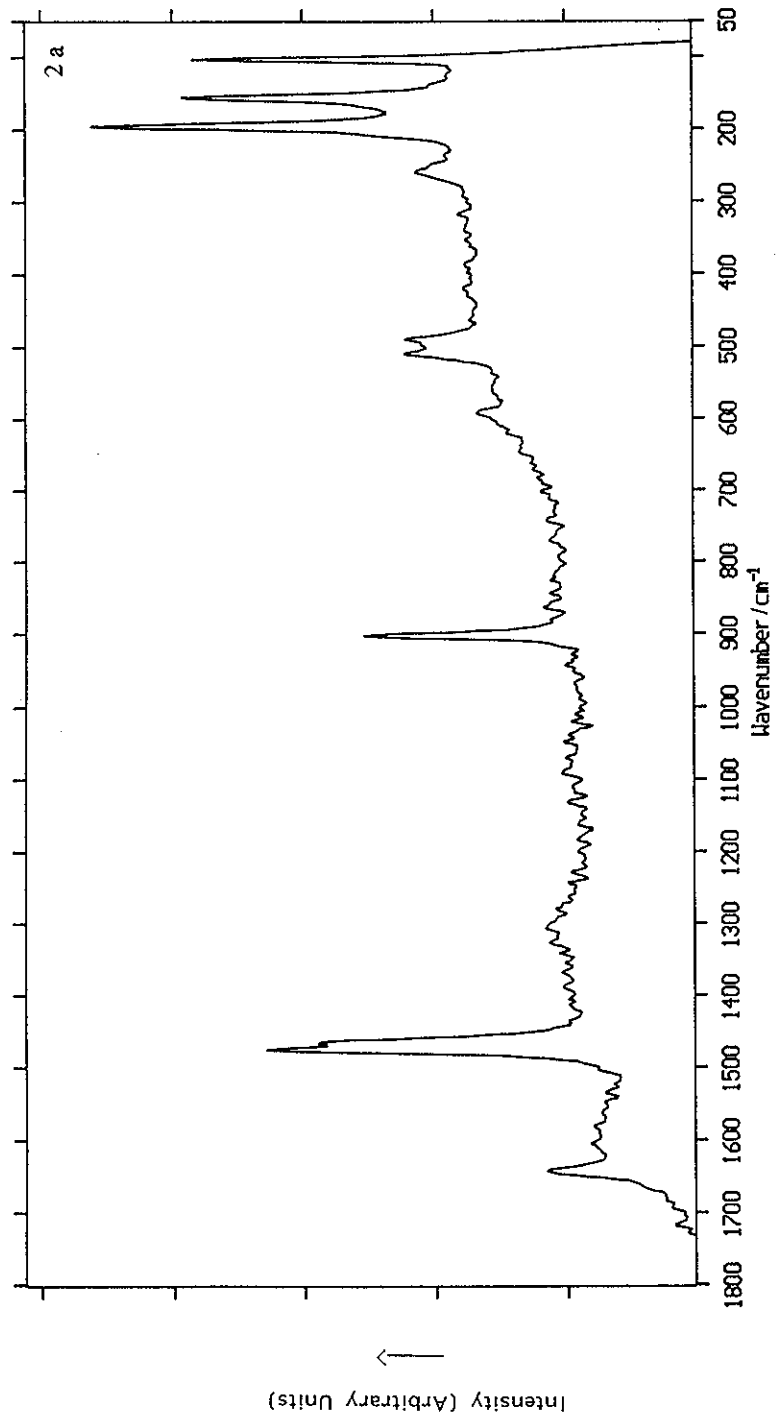


Fig. 2. Raman spectrum of *Haematoma ochroleucum* var. *porphyrium* encrustation, lower surface, 1064 nm excitation; 40x microscope objective (a) 100-1700 cm^{-1} wavenumber range (b) 2500-3400 cm^{-1} wavenumber range; resolution 4 cm^{-1} ; 16,000 scans.

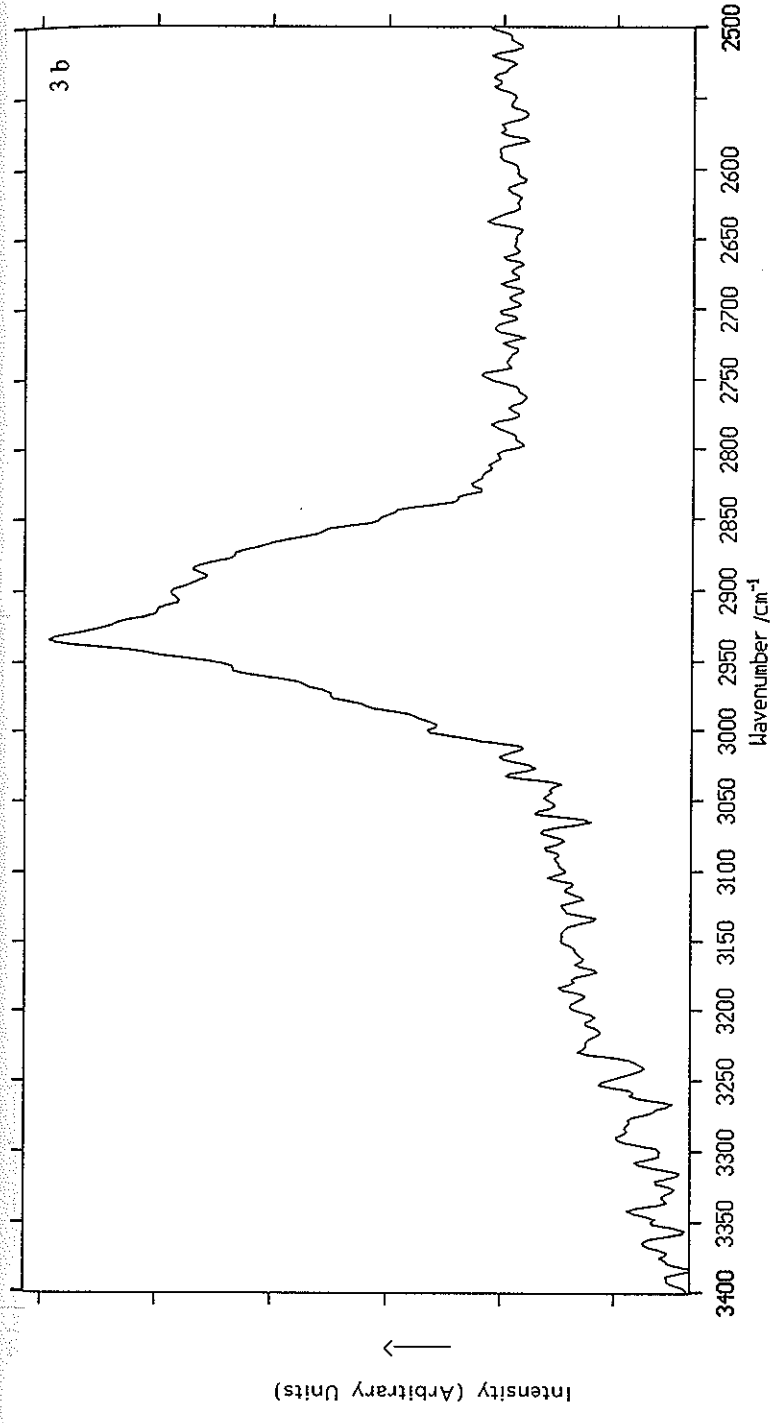
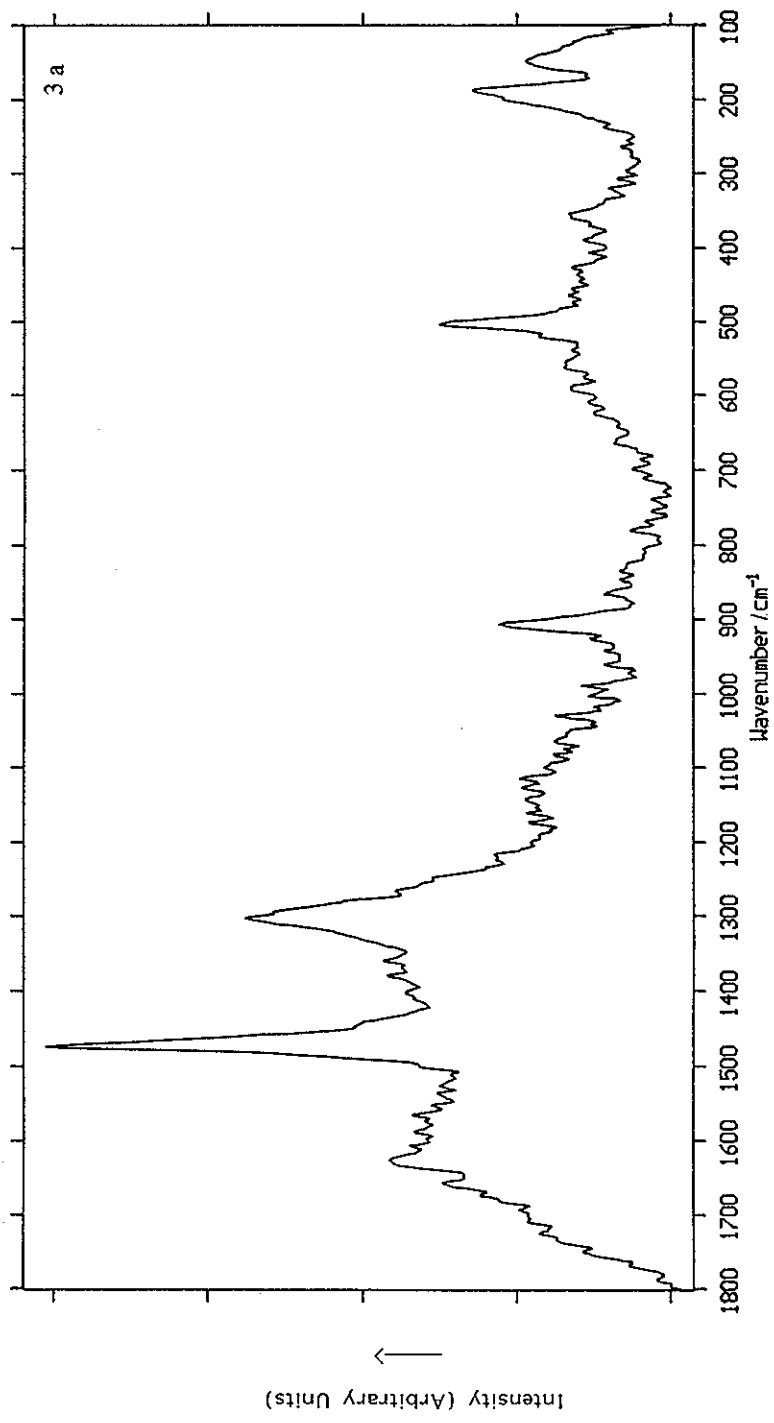


Fig. 3. Raman spectrum of *Haematoma ochroleucum* var. *porphyrum* encrustation, lower surface, white crystalline area; 1064 nm excitation, 40x microscope objective (a) 100-1800 cm^{-1} wavenumber range (b) 2500-3400 cm^{-1} wavenumber range; resolution 4 cm^{-1} ; 16,000 scans.

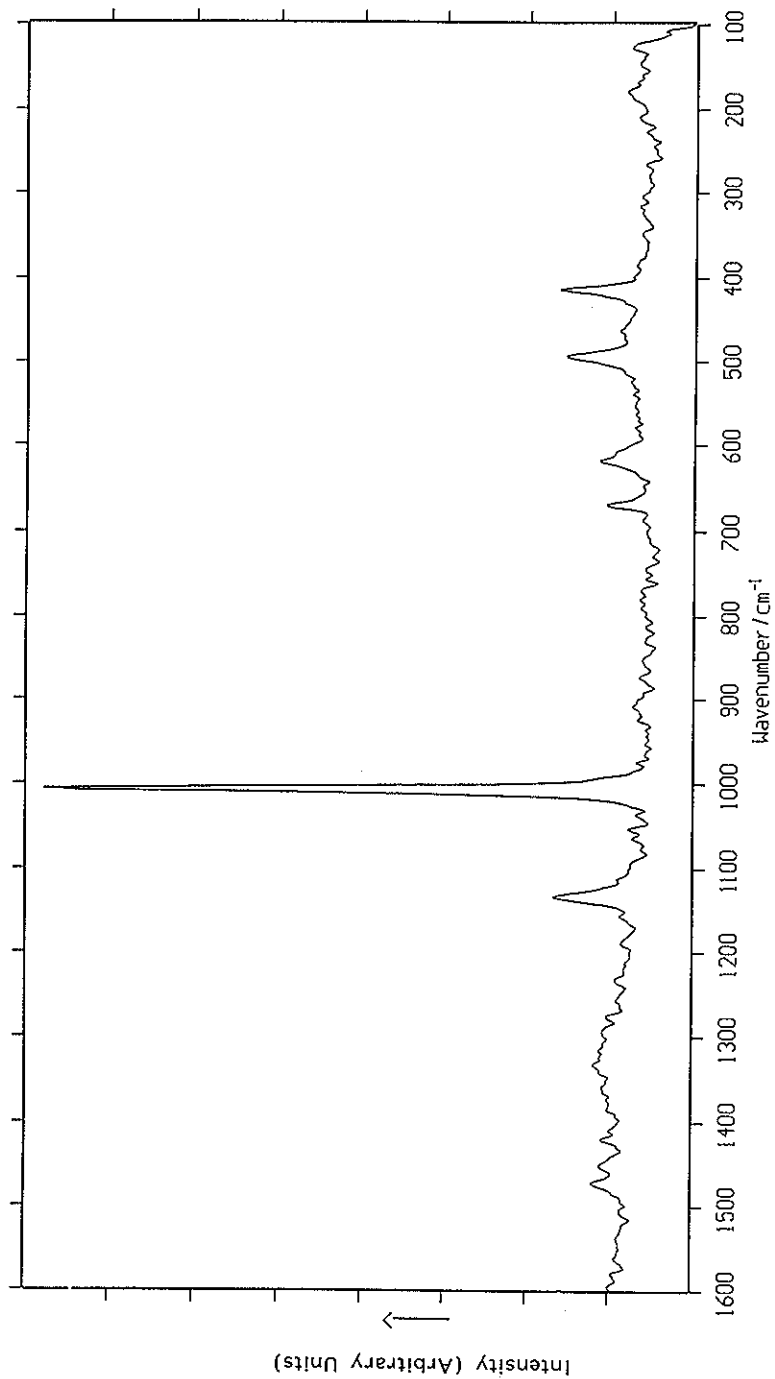


Fig. 4. Raman spectrum of particle of incorporated material on lower surface of encrustation; 1064 nm excitation; 40x microscope objective, 100-1600 cm^{-1} wavenumber range; resolution 4 cm^{-1} ; 4000 scans. The most intense feature at 1064 cm^{-1} is assignable to gypsum (calcium sulphate).

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