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COMPARATIVE FT-RAMAN SPECTROSCOPY OF *XANTHORIA* LICHEN-SUBSTRATUM SYSTEMS FROM TEMPERATE AND ANTARCTIC HABITATS

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Summary—Lichens have a primary role in the biodeterioration of rock substrata and in soil formation. In extreme Antarctic rock-face habitats, their exposed epilithic growth form makes them vulnerable to environmental stress, especially to increased UVB radiation within the late winter ozone "hole". In this study, FT-Raman spectra of intact epilithic samples of the highly-pigmented lichen species *Xanthoria elegans* and *Xanthoria mawsonii* from a continental Antarctic habitat in central Victoria Land and *Xanthoria parietina* from a maritime temperate location in Scotland have been compared. Vibrational assignments have been made for their pigments, based on parietin and an anthraquinone model. Other significant spectral features are assigned to biodegradative secondary metabolites, cellulose and incorporated substratal rock particles. Differential distribution of parietin pigment bands in the profile of an *X. elegans* thallus is probably related to the high intensity of UV-radiation reaching its Antarctic cold desert surface environment. The significant differences in Raman spectra between epiphytic *X. mawsonii* and epilithic *X. elegans* at the same site possibly indicate different biochemical UV survival strategies. In contrast to spectra from other lichen species studied in our laboratories, the *Xanthoria* encrustations contain very little calcium oxalate, formed by reaction of oxalic acid with calcareous substrata. The 462 cm^{-1} ν(SiO) band of quartz is found in the Raman spectra from both upper and lower surfaces of the lichen encrustations, confirms the physical incorporation of the substratum into the thallus.

INTRODUCTION

Many crustose lichens are highly effective colonisers and biodeteriorators of a range of rock substrata (Seaward and Giacobini, 1989a; Piervittori *et al.*, 1994, 1996). In Antarctic habitats, the exposed nature of their epilithic growth form on rocks above snow level makes them vulnerable to damage by UVB radiation, especially during stratospheric ozone depletion in late winter. Water-soluble oxalic acid produced by these usually chromogenic lichens interacts with calcium ions in rock substrata to produce insoluble calcium oxalate. The significance of calcium oxalate in its monohydrate or dihydrate forms in the weathering of calcareous rocks has been described in temperate regions (Ascaso *et al.*, 1982; Vidrich *et al.*, 1982) and the maritime Antarctic (Ascaso *et al.*, 1990). Because of their insoluble nature, oxalates tend to accumulate on the surface, within the lichen thallus and at the lichen–rock interface and leave characteristic white deposits visible (Edwards *et al.*, 1991a).

Raman microscopic analysis has proved effective in the characterization of the organic and inorganic chemical characteristics of lichen-substratum systems, particularly those involving the high-oxalate producing species, *Dirina massiliensis* f. *sorediata* (Edwards *et al.*, 1991a,b, 1992; Edwards and Seaward, 1993). In these studies, the spatial distribution and location of calcium oxalate monohydrate and dihydrate in the lichen encrustations has been determined. In more recent Raman spectroscopic studies, the hydration of the calcium oxalate deposits on substrata biodeteriorated at a variety of geographical locations and climatic sites has been addressed (Edwards *et al.*, 1997a). The effect of solar radiation exposure and temperature on *Diploicia canescens* thalli growing on limestone has been determined relative to local climatic changes in humidity (Edwards *et al.*, 1995a). Compositional information is available from infrared (IR) spectroscopic studies of similar lichen encrustations. However, the varying extent of hydration of the thallus-encrustation and the incorporation of calcareous substratal material such as calcite ($CaCO_3$)

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and gypsum ($CaSO_4$) frequently cause difficulties in interpretation of IR data (Seaward and Giacobini, 1989a; Russ *et al.*, 1995).

The biodeterioration of rock by cryptoendolithic and chasmolithic lichens in xeric Antarctic habitats is important for primary colonisation, soil formation and colonisation of new sites (Johnston and Vestal, 1989, 1993). Fourier transform (FT)-Raman spectroscopy has been used to characterise the organic composition of endolithic lichens from Victoria Land in Antarctica (Edwards *et al.*, 1997b). Cryptoendolithic communities are evident in weathered sandstone rock as coloured zones in the substratum up to 10 mm beneath the surface. FT-Raman spectra have yielded information about the role of pigmentation in lichen strategies for survival. In hostile climates, it is believed that the pigmentation of lichen thalli plays an active role in the protection of the species from UV radiation damage (Edwards *et al.*, 1995b).

To assess their potential for biodeterioration and resistance to UV damage, especially under the "ozone hole", we report the results of our FT-Raman spectroscopic studies of three highly pigmented crustose lichen species, namely *Xanthoria elegans*, *X. parietina* and *X. mawsonii*. Preliminary Raman spectra of *X. parietina* were reported in a functional study of a low-oxalate producing species occurring in a symbiotic environment with the high-oxalate producing *Ochrolechia parella* (Edwards *et al.*, 1994a). The weathering action of *X. elegans* in the Antarctic has been compared by infra-red spectroscopy and energy-dispersive X-ray analysis with a diversity of other saxicolous lichens (Ascaso *et al.*, 1990). However major advantages of the Raman spectroscopic technique used here include the absence of interference by a water signal from hydrated samples and the elimination of a need for chemical extraction or slicing into thin sections or films. The lichen need not be detached from the sample of its substratum so that examination can be conducted *in situ*. We report here a novel application of Raman spectroscopic analysis of pigments and oxalate content in *Xanthoria* species and their substrata from Antarctic and temperate habitats with widely different climatic characteristics, especially in UVB-radiation receipt under the Antarctic ozone hole.

MATERIALS AND METHODS

Samples

Xanthoria parietina (Link) Th. Fr. on sandstone substratum (Fig. 1): an orange–yellow crustose lichen was collected from a maritime coastal environment, south–west facing at sea level near Portpatrick, SW Scotland, U.K. The specimen was growing 100 m from the shore in an exposed wet position.

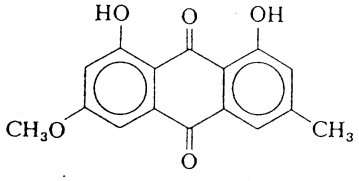
Xanthoria elegans (L.) Th. Fr.: a bright orange–red crustose lichen obtained from a sheltered east facing coastal slope (*ca.* 100 m asl) of Harrow Peaks, Victoria Land, Antarctica, was collected during a British Antarctic Survey expedition, December 1995–February 1996. The geology of Harrow Peaks (74°06′S, 164°51′E) consists of Granite Harbour Intrusives comprising two-mica granite and tonalite. The exposed granodiorite outcrops sampled for lichens faced ENE at 100 m altitude *ca.* 0.5 km from the coast of Wood Bay.

Xanthoria mawsonii: a bright red crustose lichen growing on the surface of the moss *Bryum argenteum* lithic, out of direct contact with the substratum, was collected near the source of *X. elegans* at Harrow Peaks.

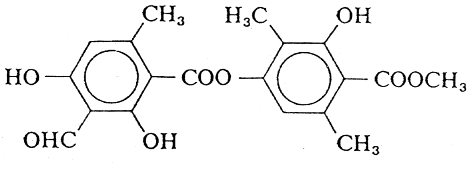
Between six and ten lichen-substratum samples were obtained for each species from their respective geographical locations. All samples were frozen to −20°C for transport and storage as soon as possible after collection.

Raman spectroscopy

FT-Raman spectra of non-dehydrated lichens were obtained using a Bruker IFS 66 infrared spectrometer fitted with an FRA 106 Raman module attachment and a Raman microscope. A Nd^{3+}/YAG laser operating at 1064 nm with a maximum source power of 400 mW was used to excite the Raman spectra; typically, only 20 mW laser power was used to record the Raman microspectra to minimise the effects of thermal heating and degradation of the sensitive biological samples. Spectra were accumulated for up to 4000 scans at 4 cm^{-1} spectral resolution. An account of the experimental



parietin



atranorin

Fig. 1. Structures of parietin, a major lichen metabolite in *Xanthoria* and atranorin (example of a polyphenolic compound)

arrangement and application of the technique to lichen examination has been given in earlier publications (Edwards *et al.*, 1997b; Wynn-Williams *et al.*, 1997; Russell *et al.*, 1998). Raman spectra were recorded from 3–6 replicate areas 100 μm in diameter from the upper and lower surfaces of each lichen thallus and of the rock substratum. The difference in wavenumber of each peak from replicate samples was negligible ($\pm$1 wavenumber) and the variation in peak height was $<10\%$. The spectra in Figs 2 and 3 are compilations of three replicate spectra. Replicate Raman spectra of the lichen-substratum profile were obtained down vertical fractures of samples.

RESULTS AND DISCUSSION

The Raman spectroscopic technique has been used in our laboratories to detect endolithic lichen pigments (Wynn-Williams *et al.*, 1997) and to distinguish between biodegradative oxalic acid and metal oxalates (Edwards *et al.*, 1991a). Chemical analysis (Culberson, 1969) of extracts of *Xanthoria elegans*, *X. parietina* and *X. mawsonii* studied here identified the major secondary metabolite parietin, and other polyphenolic compounds including erythrin, lecanoric acid and atranorin (Fig. 1). These compounds could therefore become key indicator molecules for Raman spectroscopy of specimens *in vivo*. Pure parietin is not readily available commercially, so similar compounds such as dihydroxy- and methyl-anthraquinones were used as spectral models for this material. By comparing the Raman spectrum of pure anthraquinone with spectra from a previous Raman study of substituted 1-hydroxy-9,10-anthraquinones (Dutta and Hutt, 1987), it was possible to identify the major Raman features expected for of parietin. Other compounds potentially associated with the orange and red coloration of these lichens are β-carotene and its derivatives, for which characteristic features occur at 1521 and 1157 cm^{-1}, ascribed to the ν(C=C) and ν(C–C) modes (Williams and Edwards, 1994; Veronelli *et al.*, 1995). Lichen algal (and possibly fungal) celluloses are recognisable from ν(C–C), ν(C–O) and $\delta(CH_2)$ features near 1475, 1380 and 1100 cm^{-1}. Their β-glycosidic linkage modes show around 800–900 cm^{-1} (Edwards *et al.*, 1994a,b).

Comparative FT-Raman spectra of the upper surfaces of *X. elegans*, *X. parietina* and *X. mawsonii* in the 2500–3200 and 200–1700 cm^{-1} wavenumber regions are given in Figs 2 and 3. The wavenumbers of key features and their proposed vibrational assignments are given in Table 1. *Xanthoria elegans* contains parietin, an anthraquinone, which bestows its characteristic bright orange colour. Based on comparisons with Raman spectra from anthraquinone-derivatives, the bands at 1672, 1613 and 1199 cm^{-1} were attributed to parietin in the surface of the lichen sample. Richardson (1967) found that

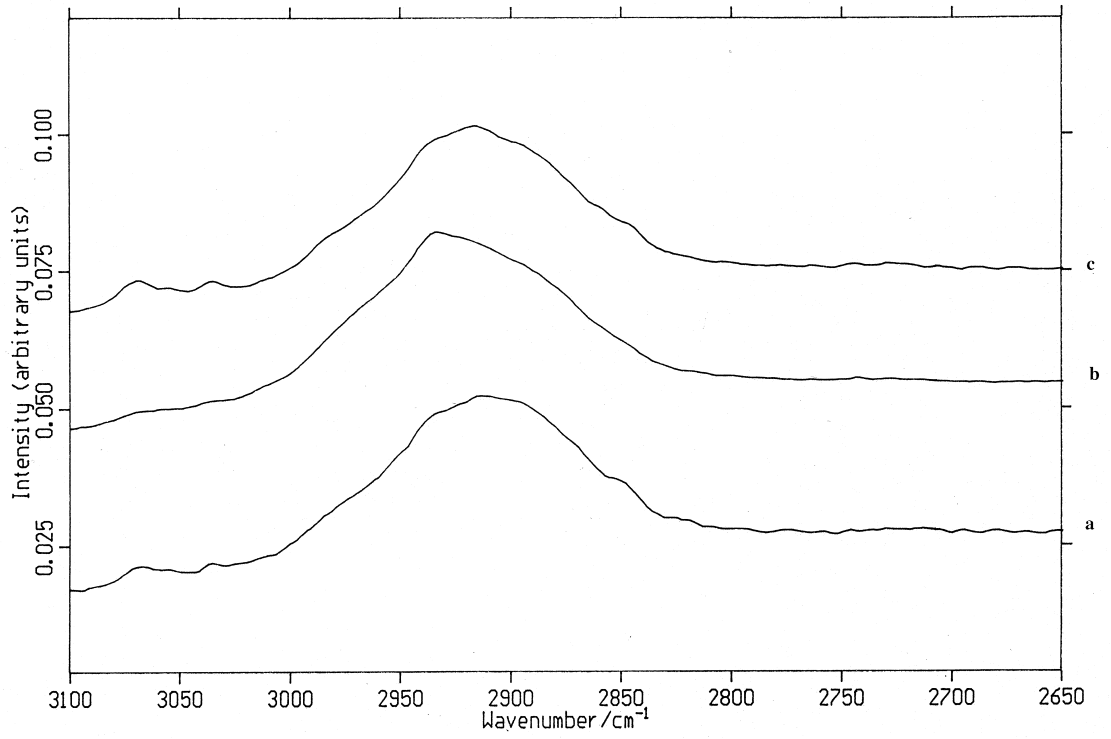


Fig. 2. FT-Raman spectra of upper surfaces of (a) *X. parietina*, (b) *X. elegans* and (c) *X. mawsonii*, 1064 nm excitation, 4 cm^{-1} resolution 4000 scans accumulated; wavenumber range 2650–3100 cm^{-1}. Peak assignments are described in Table 1

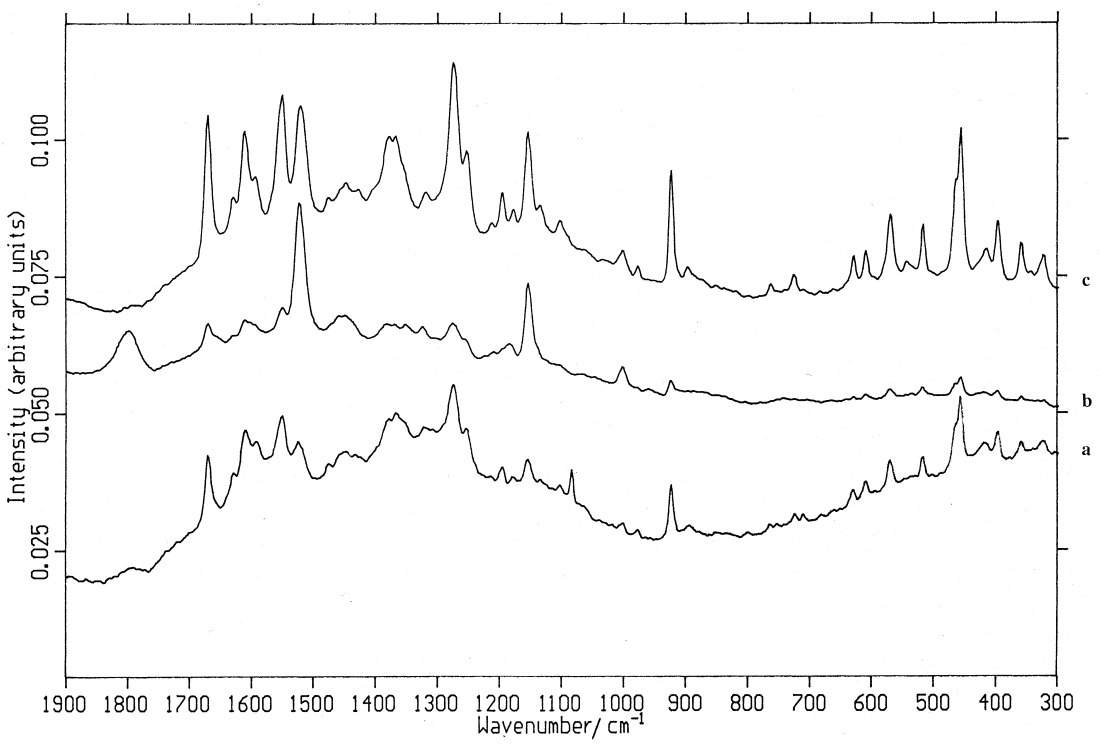


Fig. 3. FT-Raman spectra of upper surfaces of (a) *X. parietina*, (b) *X. elegans* and (c) *X. mawsonii*, 1064 nm excitation, 4 cm^{-1} resolution 4000 scans accumulated; wavenumber 300–1900 cm^{-1}. Peak assignments are described in Table 1

parietin production in *X. parietina* is under phenotypic control which suggests that it may act as an inducible UV-protectant. Both parietin and usnic acid, are common lichen substances which have strong UV-absorption peaks and are believed to have a role in UV protection (Rundel, 1978). *X. elegans* and *X. mawsoni* also show other strong pigment bands at 1521 and 1157 cm^{-1} which indicate β-carotene derivatives offering indirect protection by quenching the production of high-energy free radicals such as photo-oxidants which might damage the phycobiont photosystem (Tefler *et al.*, 1991). *X. parietina* shows the same features but of significantly lower spectral intensity.

There is little between-site variation in the intensity of the parietin bands in Raman spectra of the orange top surface of *X. elegans*. However, the yellow–grey colour of the underside suggested a lower concentration of parietin which was confirmed by very weak parietin bands at 1671 and 1613 cm^{-1} in its Raman spectrum. The bands for β-carotene at 1521 and 1157 cm^{-1} were similarly weaker in the spectrum of the underside than in that of the top surface as seen in Table 2. Raman spectra therefore indicate that the pigmentation in this lichen is concentrated at the upper surface whilst the majority of biodegradative acids produced by the lichen accumulate in the medulla. This micro-spatial information is difficult to obtain by chemical analysis and shows the merit of the Raman technique.

The Raman spectrum of the rock substratum under *X. parietina* contains a strong band at 462 cm^{-1} characteristic of α-quartz, which is a major component of sandstone (Edwards *et al.*, 1994b, 1995a,b, 1997b). This band also appears in the spectrum of the lichen surface and weakly in the spectrum of its underside which was scanned in this particular case by detaching a small specimen from the substratum. A vertical profile (*ca.* 1.4 mm in depth) through the detached specimen showed the first 0.3 mm of the orange top surface to have the same Raman spectrum as that of the surface itself. Then the pigment bands decreased rapidly in intensity towards the lower surface (Table 2). However, the Raman band for α-quartz occurs throughout the cross-section, which confirms the microscopic observation of larger crystals of substratum within the thallus. This incorporation of substratum within the thallus was reported in Antarctic lichens by Ascaso *et al.* (1982) and Walton (1993), although *X. elegans* or *X. parietina* were not included in these studies. However, the weakness in the Raman spectral bands for calcium oxalate monohydrate and dihydrate in the *Xanthoria* spp. analysed here confirm the thermogravimetric finding of Syers *et al.* (1967). They found that only 1–2% of *Xanthoria elegans* biomass was composed of calcium oxalate (and no oxalic acid) compared with 40–50% for other lichen species such as *Dirina* and *Lecanora* (Seaward *et al.*,

Table 1. Wavenumbers (cm^{-1}) and vibrational assignments for the Raman spectra of *X. elegans*, *X. parietina* and *X. mawsonii* (see Figs 2 and 3)

Xanthoria elegans	*Xanthoria parietina*	*Xanthoria mawsonii*	Proposed assignment of vibrational mode
3070 vw	3070 w	3071 w	ν(C=CH)
	3062 vw	3062 vw	ν(C=CH)
	3036 w	3936 w	ν(CH) aromatic
		2970 mw	$\nu(CH_3)$
2935 m	2938 mw, sh	2938 mw	$\nu(CH_3)$
2917 mw	2917 m	2917 m	$\nu(CH_2)$
	2892 m	2892 m	$\nu(CH_2)$
	2853 mw	2852 mw	$\nu(CH_2)$
1804 m	1795 w		ν(C=O)
1672 w	1672 mw	1672 m	ν(C=O) anthraquinone; parietin
1613 w	1613 w	1613 w	ν(CC) aromatic; parietin
	1597 w	1597 w	ν(CC) aromatic ring
1555 w	1555 m	1555 m	ν(CC) ring stretch, aromatic
1527 m	1527 mw	1527 m	ν(C=C) β-carotene
1452 w	1452 w	1452 w	$\delta(CH_2)$
1384 w	1384 m	1384 m	$\delta(CH_2)$
1369 w	1369 m	1369 m	$\delta(CH_2)$
1325 w	1325 w	1325 w	$\delta(CH_2)$; in-plane δ(OH)
1279 m	1279 w	1279 ms	$\delta(CH_2)$
1257 w	1257 w	1257 mw	$\delta(CH_2)$
–	1198 mw	1198 mw	δ(CH) aromatic ring
1155 m	1155 mw	1155 m	ν(C–C) β-carotene; anthraquinone
–	–	1136 mw	ν(COC) ring breathing
–	1086 mw	–	$\nu(CO_3^{2-})$ calcite
1003 w	1003 w	1003 w	aromatic; ν(CC) ring breathing
–	981 w	981 w	$\rho(CH_2)$
925 w	925 mw	925 m	$\rho(CH_2)$
–	897 vw	–	
–	–	768 w	ν(CC)
–	727 w	727 w	ν(CC)
–	711	–	$\delta(CO_3^{2-})$ calcite
631 w	631 vw	631 mw	δ(CCO) ring
611 w	611 vw	611 mw	δ(CCO) ring
572 w	572 m	572 m	δ(COC) ring
519 w	519 m	519 m	δ(COC) glycosidic linkage
462 w	462 m	462 ms	β-quartz
457 w	457 m	457 ms	sandstone substratum
–	413 w	413 w	
396 w	397 m	397 m	
360 w	340 w	360 mw	
–	320 w	320 mw	

s = strong; m = moderate; w = weak; sh = shoulder; v = very; ν = stretching; δ = deformation; ρ = rocking.

1989b). These results suggest that although these lichens actively biodeteriorate substrata by the physical uptake of particulate matter into the thallus, the role of oxalate in the mobilisation of minerals may be limited, albeit adequate for nutritional purposes. Cortical lichen substances other than pigments may have a secondary role in the protection of the thallus from UV radiation (Rundel, 1978). We are continuing experiments with extracted organics to quantify *better* their ultraviolet absorption.

Because of the polar amplification of UVB radiation in the Antarctic ozone hole, it is informative to compare the FT-Raman spectra of the *Xanthoria* lichen species from Antarctica (*X. mawsonii* and *X. elegans*) with *X. parietina* from a temperate climate. In Figs 2 and 3, the spectra of the ν(CH) and skeletal modes are shown. They show several significant vibrational spectroscopic differences between Polar and temperate lichen encrustations. Perhaps the most striking difference occurs in the 1500–1700 cm^{-1} region, where the relative band intensities are assigned to the quinonoid ring vibrations in

Table 2. Comparative spatial data for vertical profiles (1.4 mm depth) of lichen-substratum encrustations

Lichen	Zone	Parietin 1671 cm^{-1}	β-Carotene 1521 cm^{-1}	α-Quartz 462 cm^{-1}
X.elegans	upper	+ + +	+ +	+
	lower	+	+ +	+
	substratum	0	0	+ + +
X.parietina	upper	+ + +	+ +	+
	lower	+	+ +	+
	substratum	0	0	+ + +

Key: + + + , abundant; + + , moderate; + , present; 0, not detected.

parietin. The possible role of parietin as a UV-protector of lichen thalli has already been recognised. The Raman spectrum for *X. elegans* differs greatly in this region from that of *X. mawsonii* and temperate *X. parietina*. The *X. elegans* spectrum is dominated by the ν(C=C) pigment band at 1527 cm^{-1}, whereas the *X. mawsonii* spectrum has four features of equal intensity at 1527, 1555, 1613 and 1672 cm^{-1}. *X. parietina* has a weaker band at 1527 cm^{-1} but three strong bands at 1672, 1613 and 1555 cm^{-1}. There are strong, sharp bands in the Raman spectrum of *X. parietina* at 925 and 1279 cm^{-1} which also occur in the *X. mawsonii* spectrum but both are very weak in *X. elegans*.

The sharp feature at 1086 cm^{-1} in *X. parietina* is assigned to the (CO_3^{2-}) symmetric stretching mode of the biodeteriorated calcareous substratum and is absent from *X. elegans* and *X. mawsonii*. However, whereas all three lichen species have a weak 1003 cm^{-1} band due to an aromatic ν(CC) stretching mode, only *X. mawsonii* and *X. parietina* have a 981 cm^{-1} band, assigned to $\nu(SO_4^{2-})$ stretching and ascribed to sulphate incorporation. This is ascribed to the maritime seashore environment for *X. parietina* and *X. mawsonii*, although the period of exposure to marine influence is limited by prolonged cover of sea-ice at the coast of Harrow Peaks.

Perhaps the most significant findings for these three *Xanthoria* spp. are the differences between the Raman spectra of the two Antarctic species, despite their being collected from the same cold desert site. This suggests a diverse biochemical response to the same ecophysiological needs and is probably due in part to their different growth habits: lithic and epiphytic. Although the ν(C=C) and ν(C–C) bands of carotenoid material at 1527 and 1155 cm^{-1} are present in each to a similar extent relative to the ν(CH) band, clearly there is a deficiency in anthraquinoid content of the *X. elegans* samples.

In conclusion, the spatial distribution of a diversity of lichen bio-molecules which are potentially functional in UV-protection, biodeterioration, local nutrient-mobilisation and soil formation has been demonstrated *in vivo* using non-destructive FT-Raman spectroscopy. The characterisation of such compounds in *Xanthoria* spp. from diverse geographical locations, has potential for monitoring the effects of local environmental conditions and regional climatic change.

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