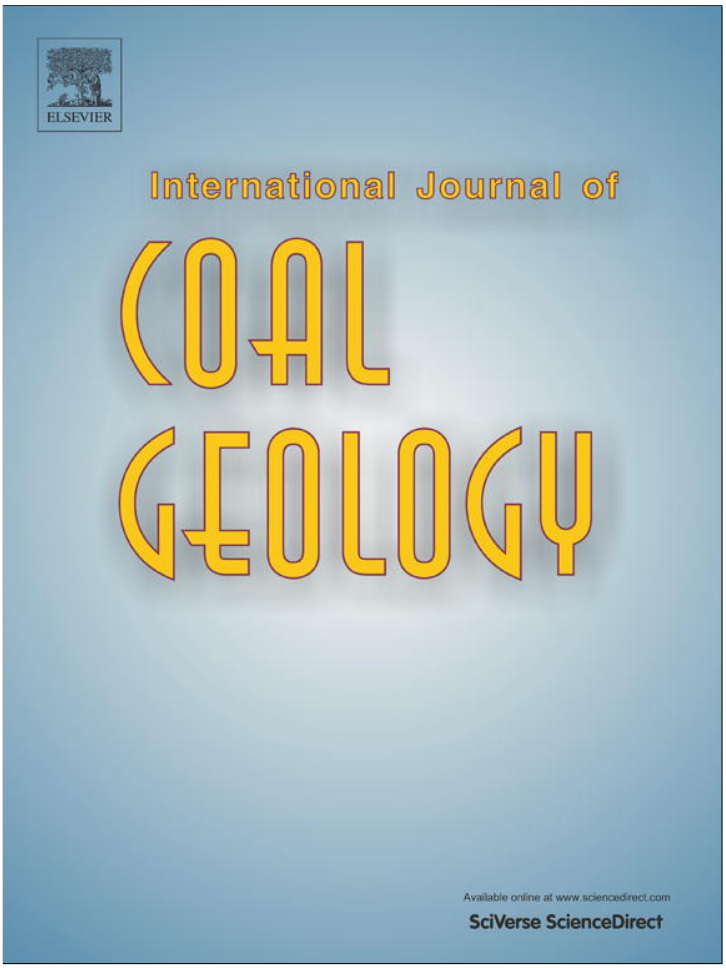

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Powder X-ray diffraction and X-ray photoelectron spectroscopy of cutin from a 300 Ma tree fern (*Alethopteris pseudograndinioides*, Canada)

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ABSTRACT

Experimental results of X-ray diffraction and X-ray photoelectron spectroscopy of fossil cutin from the compressed foliage of the Carboniferous tree fern *Alethopteris pseudograndinioides*, Cantabrian age, Sydney Coalfield, Canada, are presented in this paper. The light-colored cutin was obtained by oxidizing the compression in Schulze's solution in two stages for a total of 19 days. The broad peak in the powder diffractogram at 20° is characteristic of an average separation of ~4.4 Å between the methylenic hydrocarbon chains $(CH_2)_n$, whereas the sharper peaks at 26°–28° suggest that within a small fraction of the sample, the chains are more regularly separated. Most of the chains are likely randomly aligned to form a nematic structure.
Elemental composition by mass amounts to 58.3% C, 1.1% N, 19.4% O, 19.7% Cl, and 1.5% Si, and the local chemical environment of C 1s, O 1s, and Cl 2p is probed. Cl content is a surprising result, and further research is needed for identifying chlorine-containing species.

1. Introduction

Carboniferous cuticles offer a prime opportunity for interdisciplinary co-operation specifically in comparing taxonomy with structural chemistry. The taxonomy of the leaves (Fig. 1A to C) from Sydney Coalfield, Canada (Fig. 2A to C), and the chemistry of the cuticle/compression of *Alethopteris pseudograndinioides* via Fourier transform infrared – FTIR – have already been completed (Zodrow and Cleal, 1998; Zodrow and Mastalerz, 2007). For this study, cutin chemistry is examined via X-ray diffraction and X-ray photoelectron spectroscopy for known specimens of *A. pseudograndinioides* from Sydney Coalfield. Results are compared with those of FTIR analyses.

2. Experimental methods

2.1. Cutin and aliphatic concentration

The coalified compression was lifted from the host rock using HF, washed in distilled water, then solubilized in Schulze's oxidative solution for ca. 24 h, resulting in a hydrophobic, brownish cuticle (Fig. 3A). The cuticle was oxidized further in fresh Schulze's solution until it turned to a glittering light color, indicating cutin crystallization, which occurred after 19 days (Fig. 3B), then air-dried ready for analysis. Based on previous experiments (D'Angelo et al., 2013), the mass loss from the compression to cutin is estimated at 93%. CH_2/CH_3 ratio (FTIR determination) for the *A. pseudograndinioides* cuticle averaged 8.1 (Zodrow and Mastalerz, 2007, Fig. 10), whereas the corresponding cutin averaged 26.0. From this much longer and less branched polymethylenic side chains linked to the macromolecular cutin structure are inferred.

2.2. Powder X-ray diffraction

The crystalline cutin was evaluated through powder X-ray diffraction (XRD) using an Inel diffractometer equipped with a curved position-sensitive detector (CPS 120) and a Cu $K\alpha_1$ radiation source operated at 40 kV and 20 mA (Fig. 3C). Within the low-angle range of interest (below ~40° in 2θ), we avoided potentially overlapping amorphous peaks from the typical polycarbonate sample holder by using an aluminum-metal sample holder. This provides a low background in this angular range and has no peaks below 38.5° in 2θ. The spectrum was collected over 3 h for improved signal-to-noise ratio.

2.3. X-ray photoelectron spectroscopy (XPS)

Chemical composition was determined through XPS, which was performed on a Kratos AXIS 165 spectrometer equipped with a monochromatic Al $K\alpha$ X-ray source (14 kV, 15 mA) and a hybrid lens with a spot size of 700 μm × 400 μm. The finely ground sample was pressed

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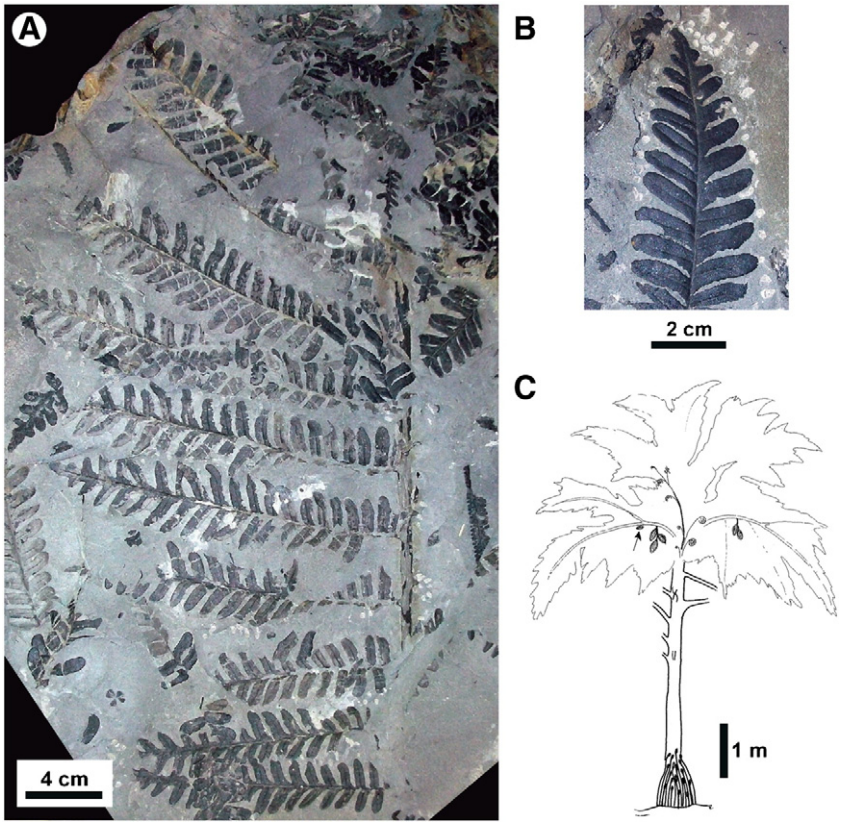

Fig. 1. Reconstruction of the *pseudograndinioides* tree-fern. (A) Detail of a large frond section. (B) Detail of the tip of an ultimate pinna. (A) and (B) are compressions. (C) Reconstructed tree-fern with ovules and male fructification (arrowed). Sources: Zodrow (2007), Cleal et al. (2010).

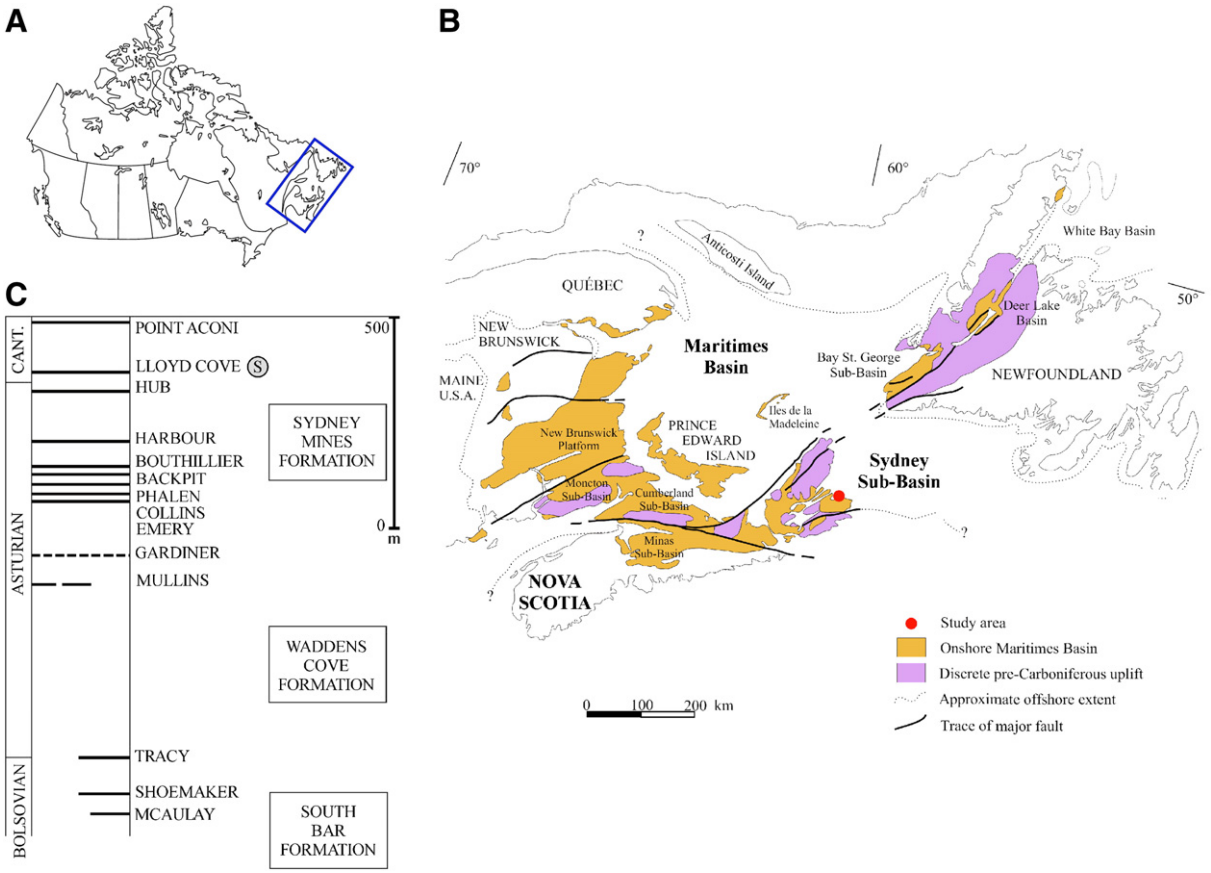

Fig. 2. (A) Canadian location map. (B) Sydney Coalfield, Nova Scotia, as part of the Maritimes Basin. (C) Coal-lithostratigraphy of the Coalfield (Bell, 1938). S = sample location at the Lloyd Cove Seam.

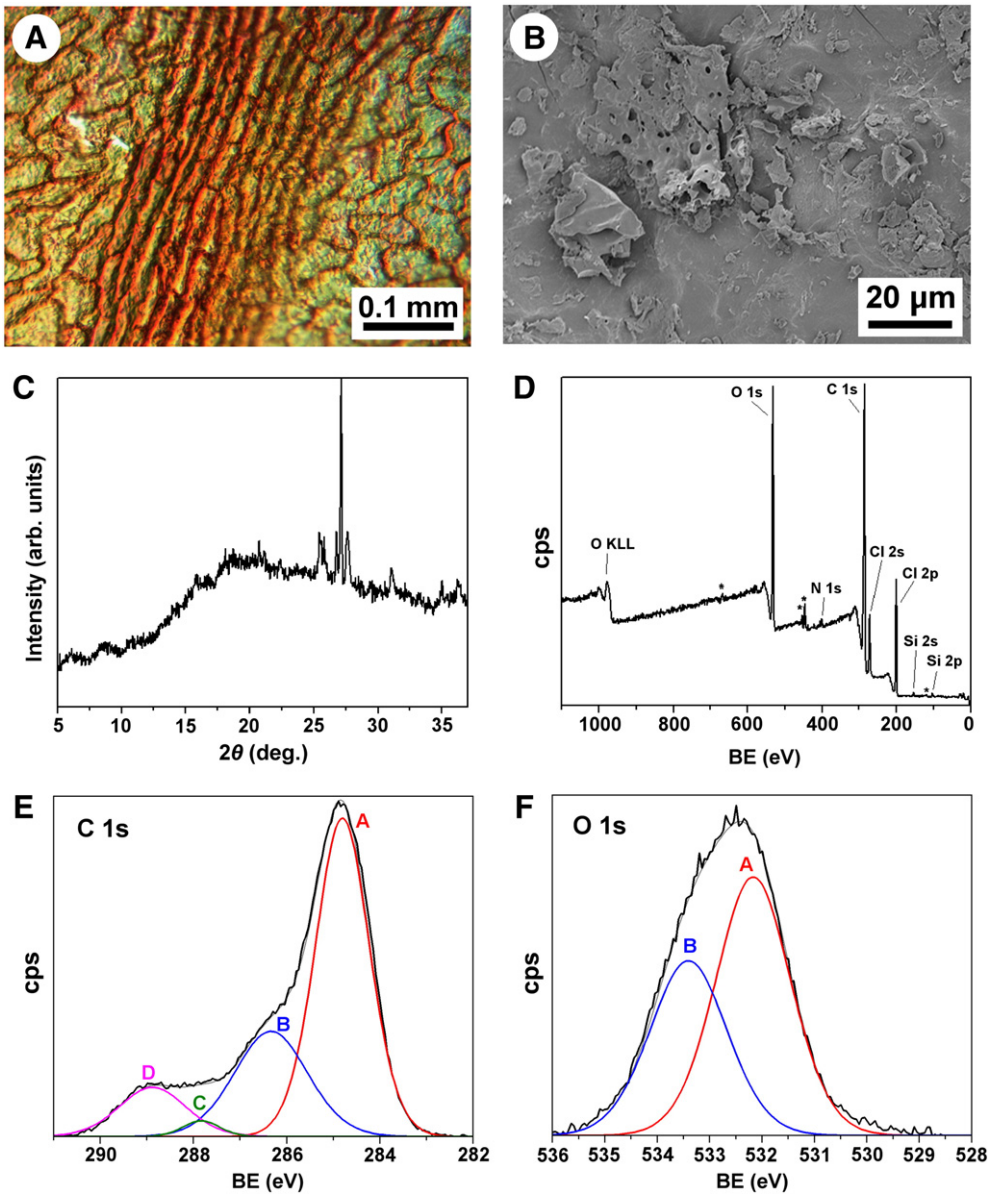

Fig. 3. *A. pseudograndinioides*. (A) Cuticle, ×125. (B) Gold-coated representative SEM micrograph of cutin with platelets? (cf. Koch and Ensikat, 2008). (C) Powder XRD pattern for cutin. (D) XPS survey spectrum of cutin, with core-line peaks assigned. Peaks originating from the indium foil used in the sample preparation are identified by asterisks. An Auger peak (O KLL) is also marked. (E) High-resolution XPS spectrum for the C 1s. (F) High-resolution spectrum for the O 1s core-line peaks. The components marked in these spectra are discussed in the text.

onto indium foil, mounted on a copper sample holder, and transferred in a sealed container to the analysis chamber of the spectrometer, within which the pressure was maintained at 10^{-7}–10^{-9} Pa. The delicate nature of the sample and its composition, rich in light elements, precluded the application of Ar^{+}-ion sputter-cleaning. A survey spectrum in the range of 0–1100 eV in binding energy (BE) was collected with pass energy of 160 eV, step size of 0.7 eV, and sweep time of 180 s. High-resolution spectra (collected with pass energy of 20 eV, step size of 0.05 eV, and sweep time of 180 s) were measured in the appropriate BE ranges for the C 1s and O 1s peaks. Because the sample is insulating, charge neutralization was applied with the best settings (charge balance of 2.0 V and filament current of 1.6 A) optimized to give the most intense and sharpest peaks. The spectra were calibrated to the C 1s line at 284.8 eV for carbon atoms bonded only to other carbon atoms, similar to the procedure used in a previous XPS analysis of apple cutin (Genet et al., 2003). The spectra were analyzed with the use of the CasaXPS software package (Fairley, 2003). The background noise due to energy loss was removed by applying a Shirley-type function and the peaks were fitted to pseudo-Voigt (70% Gaussian and 30% Lorentzian) line profiles to take spectrometer and lifetime broadening effects into account. Based on previous measurements, the precision in BE values is estimated to be ±0.1 eV.

3. Results and discussion

SEM images of the cutin sample most commonly revealed plate- and sheet-like morphologies (Fig. 3B). The thickness of these plates (~5 µm) is consistent with the expectations for cuticles of this seed-fern (Lyons et al., 1995). The three-dimensional structure of cutin is generally not well understood, and only a few X-ray diffraction studies have been made involving mimetic processes and extant fruits and leaves (Bargel et al., 2006; Heredia, 2003; Heredia-Guerrero et al., 2009; Luque et al., 1995; Reynhardt and Riederer, 1991, 1994; Rosa et al., 2012). The powder XRD pattern revealed a very broad peak that reaches its maximum near 20° superimposed by a few sharp peaks largely clustered near 26–28°, indicating the presence

of a large amorphous and a minor crystalline component, respectively (Fig. 3C). The broad peak lies well above the background that would be present in the bare aluminum metal-sample holder, whereas the sharp peaks do not correspond to any obvious inorganic residue that may have remained after a total of 19 days of oxidation of the fossil cuticle. The cuticle is composed primarily of the biopolymer cutin, which is built up of waxes, or long-chain hydroxycarboxylic acids connected through ester and other types of linkages; these chains are then assembled in parallel arrangements. The broad peak at 20° is characteristic of an average separation of ~4.4 Å between these chains, and is typically observed in the XRD patterns of other plant cutins (Heredia-Guerrero et al., 2009; Luque et al., 1995; Reynhardt and Riederer, 1991, 1994; Rosa et al., 2012). The sharper peaks at 26–28° suggest that within a small fraction of the sample the chains are more regularly separated; they correspond to *d*-spacings of 3.2–3.5 Å generated by different lattice planes (*hk*0) parallel to the chain direction, such as (110) or (200), if an orthorhombic structure is assumed (Koch and Ensikat, 2008). If the chains align in registry, a layer structure forms and long-range ordering may develop along the *c*-crystallographic direction, with long repeat distances giving rise to peaks below ~10°. Generally this alignment is imperfect, giving rise to a second broad peak at low angles as seen in some other plant cutins (Luque et al., 1995; Reynhardt and Riederer, 1991, 1994). The absence of such a peak in the present sample suggests a nematic structure in which the chains are randomly aligned along the *c*-direction.

The XPS-survey spectrum revealed, excluding hydrogen which is present but cannot be detected by this method (Fig. 3D), an elemental composition of 58.3% C, 1.1% N, 19.4% O, 19.7% Cl, and 1.5% Si by mass (atomic percent 71.9% C, 1.1% N, 18.0% O, 8.2% Cl, and 0.8% Si). The relative amounts of C, N, and O are in good agreement with cuticles of related seed ferns; in particular, the atomic O/C ratio (0.25) and C/N ratio (65.4) lie within expected ranges (Lyons et al., 1995). The presence of N may be intrinsic to the structure of cutin, perhaps in the form of amide or amine functional groups, but we cannot rule out the possibility that nitration may have occurred through reaction with nitric acid used in the maceration of the fossil (Lyons et al., 1995). The small amount of Si is attributed to residual silicates left after HF treatment to free the compression. However, the significant amount of Cl is surprising. The oxidizing agent $KClO_3$ in Schulze's solution is a possible source, but the reduced product (KCl) was not identified in the powder XRD pattern.

High-resolution C 1s and O 1s XPS spectra provided information about local chemical environments (Fig. 3E and F). The C 1s spectrum can be fit to four component peaks increasing in binding energy as the carbon centers are bonded to a greater number of electronegative substituents: peak A at 284.8 eV [C–(C, H)], peak B at 286.3 eV [C–(O, N)], peak C at 287.8 eV [O–C–O or C=O], and peak D at 288.9 eV [C–O–C=O or O=C–OH]. These results agree with FTIR spectra which reveal the presence of polymethylenic chains [$-(CH_2)_n-$], and various carbonyl (C=O) groups, i.e., in aromatic esters and carbonyl acids. The O 1s spectrum can be fit to two component peaks: peak A at 532.2 eV [C=O] and peak B at 533.4 eV [–O–]. The BEs for the C 1s peaks are consistent with those found for apple cutin, but the BEs for the O 1s peaks are about 1 eV higher (Genet et al., 2003). This observation suggests that the oxygen atoms may be bonded to an element that is more electronegative than C, perhaps a chlorate species. The BE in the high-resolution Cl 2p XPS spectrum (not shown) is 200.2 eV, which lies in the range (199–201 eV) found for many chlorine-containing compounds (Wagner et al., 2003). Further research is required to confirm if the chlorine-containing species is attributed to an unidentified residue.

4. Conclusion

The medullosalean cutin from a Carboniferous cuticle has been analyzed by X-ray diffraction and X-ray photoelectron spectroscopy for the first time. The methylenic hydrocarbon chains within the cutin are probably randomly aligned and form a thread-like structure. The elemental composition (C, N, O) is consistent with the expectations for this cutin, but the presence of Cl requires further investigation. The local environments of C and O, as probed by high-resolution XPS spectra, correspond to functional groups (i.e., methylenic chains, carbonyl groups) previously inferred from FTIR spectra.

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