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PRESERVATION OF PLANT CUTICLES

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ABSTRACT. Recent and fossil conifer and *Ginkgo* cuticles have been studied using a combination of SEM, TEM, py-GC-MS and FT-IR. Results show that a highly resistant original chemical composition is not a prerequisite for preservation. Selective preservation (of a resistant macromolecule such as cutan) or random repolymerization cannot explain the preservation of cuticles in fossil leaves of conifers or *Ginkgo*. Cuticle preservation as a resistant organic fossil is considered to result from formation of a macromolecular matrix by within-cuticle diagenetic stabilisation of normally labile constituents.

KEY WORDS: cuticle, conifer, Ginkgo, macromolecule, preservation, fossil

INTRODUCTION AND OBJECTIVES

This paper is a short, botanically orientated review of studies of Recent and fossil plant leaf cuticles considering the factors which might explain preservation (or loss) resulting in a biased fossil record. We aimed to determine the significance of original chemical composition, presence/absence of resistant macromolecules, parent plant, enclosing rock, thermal history and geological age. Our approach combined evidence from morphology, ultrastructure, chemical composition and short term natural decay experiments.

The base material of recent plant cuticular membranes is a cross-linked polyester, cutin, derived from families of C_{16}- and C_{18}- hydroxy-carboxylic acids along with lipids and/or waxes (Holloway 1982, 1984). Cuticles of the Recent angiosperm *Agave* contain a more resistant macromolecular material, named cutan, studied first by Nip *et al.* and Tegelaar *et al.* (see refs in van Bergen *et al*. 1995, Mösle *et al*. 1997). Pyrograms of *Agave* cuticle show prominent doublets of straight chain aliphatic hydrocarbons. In this feature, pyrograms of fossil cuticles (Fig. 1b, d; Collinson *et al.* 1994, 1998, Van Bergen *et al.* 1995, Mösle *et al*. 1997, 1998, Stankiewicz *et al*. 1998) resemble *Agave*, supporting the suggestion that selective preservation of cutan might be involved in preservation of fossils (Tegelaar *et al*. 1991). An alternative model for preservation of organic matter invokes random repolymerization (Tissot & Welte 1984), in which case all fossil organic matter in a given sample would be expected to show similar chemistry.

MATERIAL

Conifers and *Ginkgo* (maidenhair trees) were selected for this study as they are considered by some authors (Beck 1988 p. 452) to represent a monophyletic group of seed plants and they also have an excellent fossil record (Beck 1988). In addition there are close relatives (cordaites, sister group to conifers) and more distant (pteridosperms) extinct relatives in the Carboniferous (Beck 1988) which we have also studied (Collinson *et al*. 1998, Stankiewicz *et al*. 1998). The cutan-containing Recent flowering plant *Agave americana* was studied for comparison. In all we have studied Recent *Ginkgo*; 15 genera from 6 families (Kubitzki 1990) of Recent conifers; two Tertiary conifers; several genera from extinct (Palaeozoic and Mesozoic) conifer families; Palaeozoic cordaites and pteridosperms; as well as fossil *Ginkgo* spanning Liassic to Palaeocene in age. Results have been published (Mösle *et al*. 1997, 1998, Collinson et al. 1998) and we also have unpublished data on Mesozoic and Cainozoic conifers (Cheirolepidiaceae, Taxodiaceae, *Sciadopitys* and extinct relatives of *Ginkgo*); Recent *Agave*; and Recent Taxodiaceae, Pinaceae and Sciadopityaceae.

METHODS

Modern cuticles were released from the leaf using hydrogen peroxide and glacial acetic acid. Fossil cuticles were physically picked from the rock or released using HCl + HF. All cuticles were lipid/solvent extracted prior to analysis. Modern cuticles were subjected to selective chemical extraction (acetylation and saponification) to distinguish specific components. The morphology and ultrastructure of the cuticles were monitored by scanning and transmission electron microscopy (SEM & TEM). Cuticles were analysed using pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) and Fourier Transform Infrared spectroscopy (FT-IR). Details of methods are given in Collinson *et al*. (1998) and Mösle *et al*. (1997).

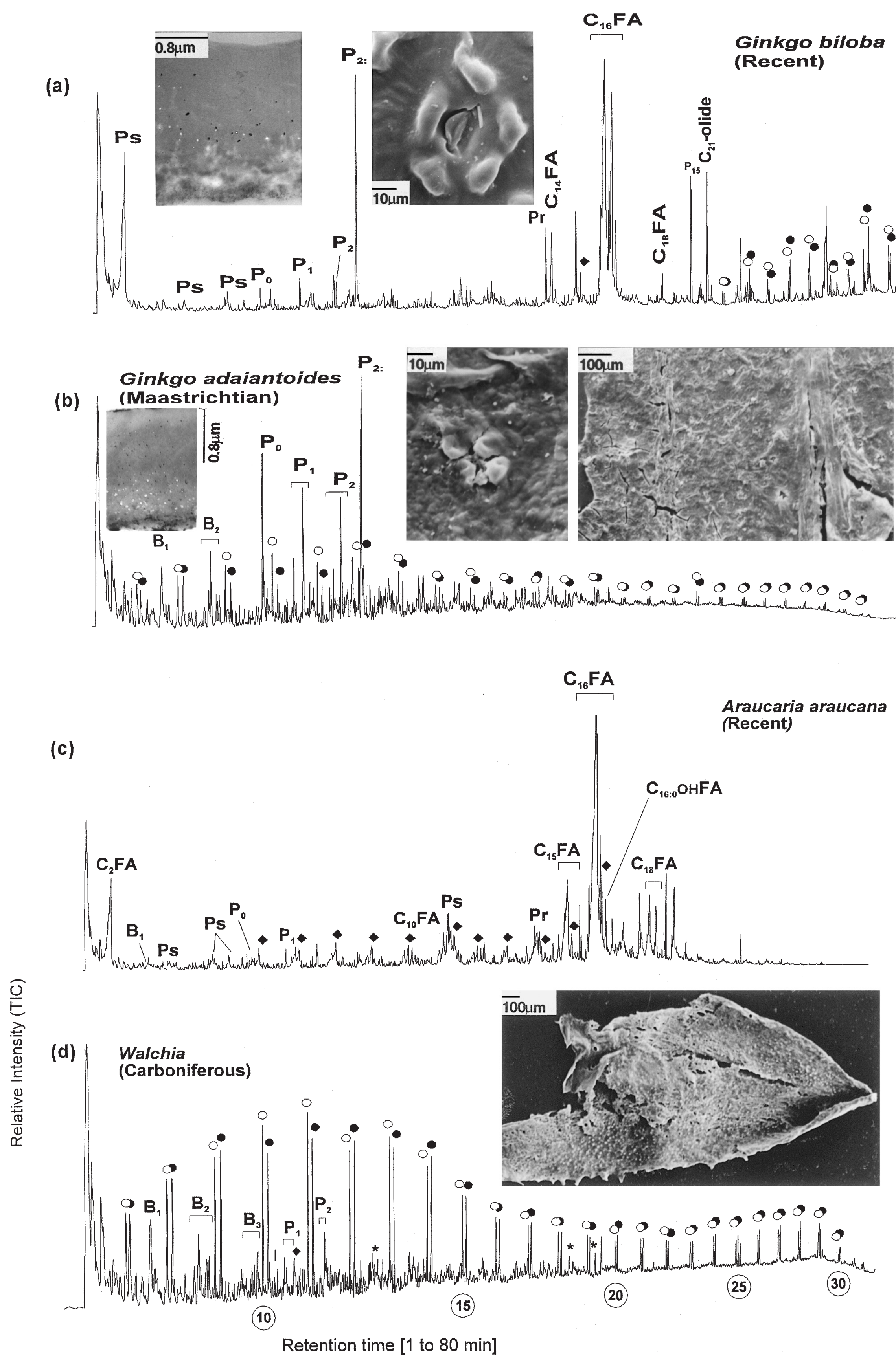

Fig. 1. Recent (a, c) and fossil (b, d) leaf cuticles of *Ginkgo* (a, b) and conifers (c, d). Line figures show total ion chromatograms of pyrolysates (CDS at 610°C for 5s). Peaks: – FA=cutin-derived, P=phenolic, B=benzenoid, Ps=polysaccharide-derived, ml=homologous series of alkene/alkane hydrocarbon series doublets, u=methylketones. In *Ginkgo* SEM of stoma (centre) and TEM (left) show retention of morphology and ultrastructure in fossils. SEM's (far right) show that fossil cuticles retain morphology as large sheets (*Ginkgo* with two veins and intervein areas) or complete leaves (*Walchia*, scale-like leaf)

RESULTS

MODERN CUTICLES

Sequential chemical extraction (Mösle *et al*. 1997) successfully isolates cuticle components (proven by py-GC-MS, SEM & TEM); acetylation removes polysaccharide and lignin cell wall components whilst saponification removes cutin. A substantial (approx. 15% by weight), recoverable cutan residue remains from *Agave* after both processes. Cuticles of Recent *Ginkgo* and conifers do not yield a resistant residue (max. 2%) after saponification and acetylation. Pyrograms (Fig. 1a, c) and IR traces of *Ginkgo* and conifer (e.g. *Araucaria* shown here) cuticles show that they consist mainly of cutin indicated by cutin fatty acid monomers (FA) in pyrograms and aliphatic (C-H), hydroxyl (O-H) and ester carbonyl (C=O) functions in IR spectra. Fragments derived from pyrolysis of polysaccharides (Ps, mostly furan derivatives) are probably from attached cell wall material.

Cuticle preparations differ, the presence of cutin polyester is universal but morphology and other constituents vary. Pinaceae "cuticles" included epidermal cell walls plus two layers of hypodermal fibres. Taxodiaceae 'cuticles' included all the epidermal cell walls whilst *Ginkgo* 'cuticles' (and *Agave*) included only the outer periclinal epidermal cell wall which was often partially detached. SEM & TEM monitoring (Fig. 1a) combined with selective chemical extractions was essential to establish what was being analysed.

Short term natural decay (up to 30 wks in pond sediment) resulted in minimal changes in pyrolysates. EM showed disruption beginning in the cell wall / cuticle intergradation zone. These results have been published in full elsewhere (Collinson *et al*. 1998).

FOSSIL CUTICLES

Electron microscopy shows that fossil cuticles can be large sheets or complete leaves, essentially unaltered, retaining characteristic morphology and ultrastructure (EM's Fig. 1b, d). Nevertheless their chemical character has been drastically changed (Compare pyrograms Fig. 1a, c with Fig. 1b, d).

Py-GC-MS (Fig. 1b, d) of fossil *Ginkgo* and conifers (e.g. *Walchia* shown here) show loss of cutin fatty acid monomers (FA) and of polysaccharide pyrolysis products (Ps). Instead there is a characteristic aliphatic pattern with an homologous series of alkene/alkane (ml) hydrocarbon series extending up to the GC limit (C_{35}) in some cases. The hydrocarbon profile can vary considerably. Benzenoid (B) and phenolic (P) compounds can be present but the identities and the amounts vary . IR spectra show retention of aliphatic (C-H), hydroxyl (O-H) and carbonyl (C=O) functions. However, the aliphatic C-H content diminishes progressively with age and the ester carbonyl is transformed to carboxylic acid or ketone groups (Collinson *et al*. 1998).

All fossil *Ginkgo* and conifer cuticles show the same fundamental alterations to their chemistry irrespective of the type of plant, cuticle morphology and ultrastructure, enclosing lithology, thermal maturity or geological age (Mösle *et al*. 1997, 1998, Collinson *et al*. 1998).

In detail of their chemical character, pyrograms show greater similarity between related fossils, e.g. two cordaites, from different rock samples, than between different cuticles, e.g. cordaite/pteridosperm, from the same rock sample (Stankiewicz *et al.* 1998). Pyrograms of fossil conifers differ from those of fossil *Ginkgo*, which themselves share a common signature (Mösle *et al*. 1998). Fossils thus retain a chemical signature (albeit modified) from the original cuticle, dependent on the type of source plant.

CONCLUSIONS

SEM alone is inadequate to visualise and monitor the effects of chemical treatments on cuticles. SEM & TEM combined are essential. SEM, TEM, pyrolysis and IR spectroscopy give complementary information, the use of one technique alone can be misleading with regard to 'cuticle' composition. The chemical changes in fossils evident from pyrolysis (incorporation of hydrocarbon chains longer than cutin C_{16}/C_{18}) and infra red (retention of C=O but alteration of chemical environment, relative decrease in aliphatic C-H content with age) can take place with minimal changes to morphology.

A highly resistant original chemical composition is not a prerequisite for preservation of leaves as fossils because conifers and *Ginkgo* (which lack highly resistant chemistry) are common fossils. Selective preservation of a resistant macromolecule (such as cutan) cannot explain the preservation of cuticles in fossil leaves of conifers or *Ginkgo*. Retention of morphology, ultrastructure and cuticle specific chemical signatures, including in co-occurring animal and plant cuticles (Stankiewicz *et al*. 1998), shows that chemical alterations in fossils cannot be due to random repolymerisation of organic matter in the sediment.

Taking into account the full results of our work published elsewhere (Mösle *et al*. 1997, 1998, Collinson *et al*. 1998) we deduce that cuticle preservation as a resistant organic fossil results from formation of a macromolecular matrix by within-cuticle diagenetic stabilisation of normally degradable aliphatic constituents. Chemical evidence suggests replacement of ester links by other carbonyl functions and stabilisation of aliphatic chains; the longer chains may include contributions from entrained and surface waxes.

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