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Biomarker compositions of *Glyptostrobus* and *Metasequoia* (Cupressaceae) fossils from the Eocene Buchanan Lake Formation, Axel Heiberg Island, Nunavut, Canada reflect diagenesis from terpenoids of their related extant species

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ABSTRACT

The solvent extractable organic compounds of two fossil conifer species from the Eocene Buchanan Lake Formation, Axel Heiberg Island, Canada, were compared to the compound composition of related extant species to investigate the preservation and degradation (diagenesis) of terpenoid biomarkers in geological samples. Mono-, di- and triterpenoids, lignin components and aliphatic lipids were identified in the solvent extracts of the fossil and extant conifers. The biomarker composition of fossil *Glyptostrobus* shoots and seed cones is different from their pattern observed in fossil *Metasequoia*. The comparison of terpenoids found in fossil plant extracts with those present in their related extant species shows similar patterns of terpenoid biomarkers and confirms the systematic assignment of fossil species based on morphological and anatomic characteristics. The presence of unaltered natural product terpenoids and their only slightly altered diagenetic derivatives (biomarkers) reflect the excellent preservation of the Eocene fossils from the Buchanan Lake Formation.

1. Introduction

Terpenoids in conifer resins are useful biomarkers for the chemosystematic assignment of extant conifer families and genera (e.g. Erdtman and Norin, 1966; Hegnauer, 1962, 1986; Gadek and Quinn, 1985; Otto and Wilde, 2001). Terpenoids have also been reported from fossil conifer remains such as wood, shoots and cones (e.g. Otto and Simoneit, 2001; Otto et al., 2002, 2003; Staccioli et al., 2002a,b; Marynowski et al., 2007) due to their excellent preservation potential in sediments and plant fossils. The direct comparison of terpenoids in fossil plants with the resin biomarkers of their respective extant relatives is therefore an excellent tool for the chemosystematic assignment of fossil species and the study of the geochemical degradation of biomolecules (Otto et al., 2002, 2003, 2007).

Predominantly deciduous forests, with a significant element of deciduous conifers, existed north of the Arctic Circle during the Mid Eocene (~45 Ma), and at the Fossil Forest site of the Buchanan Lake Formation of Axel Heiberg Island, Canada. The remains of these forests are exceptionally well preserved (e.g. Basinger, 1991; Greenwood and Basinger, 1994; McIver and Basinger, 1999). These forests flourished in the region (~78°N) during a summer that included three months of continuous light, and were efficiently dormant during the three months of darkness in winter (Jahren, 2007). Paleoenvironmental reconstruction of the locale has indicated an ice-free, warm climate with high precipitation during the growing season (Jahren, 2002, 2003, 2008; Greenwood et al., 2010; Schubert et al., 2012; West et al., 2015). The sediments represent fluvial and lacustrine deposits containing in situ fossil forests commonly dominated by *Metasequoia*. Fossil remains such as leaves, conifer cones and resinites are preserved as forest floor litter within uncemented silt to fine sand and show little diagenetic alteration, a condition called mummification. Therefore, the fossils show excellent preservation of morphological and anatomical structures.

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Fossil wood of similar age and preservation, with intact cellular structures and resin, has been recovered from a kimberlite pipe (~53 Ma) in the Slave Province (~64°N presently), Northwest Territories, Canada, and analyzed by stable isotope methods (Wolfe et al., 2012). Based on the wood anatomy the sample was identified as *Metasequoia*, a dominant forest species in the late Paleocene through the Eocene at these northern latitudes (Sunderlin et al., 2011; Wolfe et al., 2012). The stable isotope data support the conclusion that the *Metasequoia* forests of the subarctic latitudes of Slave Province grew under paleoclimates 12–17 °C warmer and much wetter than presently (Wolfe et al., 2012).

Earlier studies of the biogeochemistry of Axel Heiberg fossils include solvent extractable biomarkers of resinites and fossil wood and pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) of resinites and cones. Solvent extractable biomarkers of several wood and bark remains have been reported by Obst et al. (1991) and Staccioli et al. (2002a,b). Resinites associated with fossil seed cones of *Metasequoia* Miki (Cupressaceae s. l.), *Pinus* L. and *Pseudolarix* Gordon (both Pinaceae) from the Eocene of Axel Heiberg Island, Canada, have been analyzed by Py-GC–MS, and it was speculated that *Pseudolarix* and the succinite producing species share a common ancestor (LePage and Basinger, 1991; Anderson and LePage, 1995). Here we report for the first time the solvent extractable biomarkers (terpenoids and lipids) of shoots and seed cones of the fossil conifers *Glyptostrobus* (*G. nordenskioeldi* (Heer) Brown) and *Metasequoia* (*M. occidentalis* (Newberry) Chaney) from the Buchanan Lake Formation of Axel Heiberg Island, and compare them to the natural bioterpenoids in their related extant conifer species.

2. Samples and methods

2.1. Samples and geological setting

Specimens of fossils, as well as extant species *Glyptostrobus pensilis* (Staunton ex D. Don) K. Koch and *Metasequoia glyptostroboides* Hu et Cheng, were analyzed (Table 1). These genera were formerly assigned to the Taxodiaceae, but have since been incorporated into the Cupressaceae sensu *lato* (*s. l.*) (e.g. Gadek et al., 2000).

The fossil samples were recovered from sediments of the Buchanan Lake Formation, Eureka Sound Group, Axel Heiberg Island, Nunavut, Canadian Arctic Archipelago (79°55′N, 89°02′W) (Ricketts, 1986, 1991, 1994; Basinger, 1991; Francis, 1991; Basinger et al., 1994; Williams et al., 2003; Jahren, 2007; Liu and Basinger, 2009). Locality numbers UofS 184, 220, and 236 in Table 1 are site designations from the University of Saskatchewan Paleobotanical Collection (see McIver and Basinger, 1999 for additional information). An age of Middle Eocene has been established based on the regional context, palynology and paleofloristics, and vertebrate paleontology (see Ricketts, 1994; Basinger, 1991; McIver and Basinger, 1999; Eberle and Storer, 1999). The anatomically and morphologically well preserved (mummified) fossil shoots and seed cones have been subject to little diagenetic or metamorphic alteration. They were removed from the silt sediments, quickly air-dried and stored in teflon bags. The dried samples were crushed in a mortar prior to extraction.

Samples of seed cones (dry), bled resin, and green leaf shoots from extant *Glyptostrobus pensilis* and *Metasequoia glyptostroboides* were collected from living trees in China (Table 1). The bled resins had exuded from the trees after pruning or insect damage. The seed cones were cut into small pieces with a scalpel prior to extraction; the resin was directly dissolved in organic solvents, and the shoots were dipped briefly in *n*-hexane.

2.2. Extraction and derivatization

The pulverized fossil samples were ultrasonicated three times for 10 min each with dichloromethane:methanol (DCM:MeOH, 1:1; *v*/v). The cut cones of the extant species were sonicated for 10 min with DCM:MeOH (1:1; v/v) and then soaked in the solvent mixture for 24 h. The resin was dissolved completely in DCM:MeOH (1:1; v/v). All solvent extracts were filtered through glass fiber filters, concentrated to 500 μL by use of a rotary evaporator and then under blow-down with dry nitrogen gas. Aliquots (50 μL) of the total extracts were converted to trimethylsilyl derivatives by reaction with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine for 3 h at 70 °C. Prior to analysis the excess silylating reagent was evaporated and the sample mixture dissolved in an equivalent volume of *n*-hexane. Other aliquots (50 μL) in DCM:MeOH (1:1, v/v) were treated with trimethylsilyldiazomethane to methylate carboxylic acids prior to analysis. This reaction proceeds within 30 min at room temperature after which the excess reagent is removed with acetic acid, followed by blow down and redissolution in *n*-hexane.

2.3. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analyses of the total extracts and of aliquots of the derivatized total extracts (typical injection volume 1 μL) were performed on a Hewlett-Packard model 6890 GC coupled to a Hewlett-Packard model 5973 MSD. Separation was achieved on a fused silica capillary column coated with DB5-MS (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC operating conditions were as follows: temperature hold at 65 °C for 2 min, increase from 65 to 300 °C at a rate of 6 °C min^{-1}, and final isothermal hold at 300 °C for 20 min. Helium was used as carrier gas. The sample was injected splitless with the injector temperature at 300 °C. The mass spectrometer was operated in the electron impact mode at 70 eV and scanned from 50 to 650 Da. Data were acquired and processed with the Chemstation software. Individual compounds were identified by comparison of mass spectra and retention times with those of authentic standards, with literature and library data, and interpretation of mass spectrometric fragmentation patterns of unknowns.

3. Results

The dominant component classes in the extracts of the fossil and extant conifer species analyzed here are diterpenoids, aliphatic lipids, triterpenoids, steroids, and lignin-derived phenols (Table 2 all structures of listed compounds are given in Appendix A). The

Table 1
Description of fossil and extant conifer samples.

Species	Sample	Origin	Age
Glyptostrobus nordenskioeldi	Seed cones	Axel Heiberg Island, location UofS 220	Eocene
Glyptostrobus nordenskioeldi	Shoots	Axel Heiberg Island, location UofS 220	Eocene
Glyptostrobus pensilis	Bled resin, seed cones and shoots	Botanical Garden of the Chinese Academy of Sciences, Guangzhou, China	Extant
Metasequoia occidentalis	Seed cones	Axel Heiberg Island, location UofS 184	Eocene
Metasequoia occidentalis	Shoots	Axel Heiberg Island, location UofS 236	Eocene
Metasequoia glyptostroboides	Bled resin, seed cones and shoots	Botanical Garden of the Chinese Academy of Sciences, Beijing, China	Extant