

Confined pyrolysis of extant land plants: A contribution to palaeochemotaxonomy

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Abstract

In order to aid the interpretation of plant biomarker assemblages found in the geological record in terms of palaeoflora composition, a new experimental approach, based on artificial maturation of extant plants using confined pyrolysis was developed. A representative Pinaceae species (*Abies pinsapo*) was selected for study because these plants synthesize large amounts of abietanoic acids for which the diagenetic pathway is well known. Pyrolysis parameters were defined so as to allow the formation of all the abietanoic acid-derived diagenetic products encountered in ancient sediments. With pyrolysis parameters of 700 bar, 24 h and 280 °C, partial degradation of dehydroabietic acid leads to the formation of all the typical aromatic abietanes (e.g. simonellite, retene, etc.) found in the geosphere. Synthesis of saturated abietanes (norabietanes, abietanes) was activated with the pyrolysis of a mixture comprising a reducing agent (LiAlH₄) and the plant material. We also show that the distribution of plant biomarkers formed under these two pyrolysis conditions is consistent with those of the fossil Pinaceae in published palaeochemotaxonomic studies. Consequently, the procedure could be extended using a broad range of plant taxa in order to predict their fossil molecular signatures. New palaeochemotaxonomic trends for other taxa could therefore be found. In the future, the data thus gathered could not only be useful for the reconstruction of palaeoflora populations and palaeoclimates but also for archaeological and other research areas.

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1. Introduction

Vascular plant biomarkers are frequently found in sediments and sedimentary rocks deposited in marine and terrestrial environments. These bio-

markers derive from the diagenesis of compounds biosynthesized by land plants following deposition in sedimentary basins. Many studies of the chemical composition of terrestrial plants highlight the chemotaxonomic value of numerous biological compounds, meaning that they are synthesized by a restricted number of taxa (e.g. Aplin et al., 1963; Smith, 1976; Castro et al., 1996; Mongrand et al., 2001). During diagenesis, some of these biological

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compounds, mainly terpenoids, are degraded to become geoterpenoids and can retain their initial chemotaxonomic value (Otto et al., 2002a). The relationship between the biomarker composition of fossil organisms and the systematic classification of living organisms is studied using palaeochemotaxonomy. For instance, Cupressaceae, a conifer family, is believed to be the unique taxon able to biosynthesize significant amounts of cuparene class sesquiterpenoids such as cuparenic acid. During diagenesis, cuparenic acid is degraded to cuparene which is thus considered as a specific biomarker for ancient Cupressaceae (Grantham and Douglas, 1980).

In theory, it is possible to link many specific markers to ancient organisms. Thus, the distribution of vascular plant biomarkers preserved in sedimentary rocks is a proxy for terrestrial plant assemblages constituting the palaeoflora. Furthermore, as palaeoflora changes are under climatic control, vascular plant biomarkers can be used as a chemostratigraphic tool for tracing both palaeoflora and palaeoclimatic changes over geological time, from the colonization of terrestrial environments by plants during the Silurian to the Quaternary (Vliex et al., 1994; van Aarssen et al., 2000; Hautevelle et al., 2006).

Palaeobotany and palynology are classically used for the reconstruction of ancient flora assemblages and palaeoclimates (Barale, 1990; Vakhrameev, 1991; Ziegler et al., 1994). However, these approaches, although complementary, provide only partial information because of:

- (1) The difficulty in associating fossils, spores and pollen to precise taxa with certainty. Indeed, fossil wood and spore pollen are usually described as xylomorphs and sporomorphs, respectively, and their relation to the systematic classification of plants often remains unknown, especially for pre-Cenozoic times. Furthermore, palynology is more commonly used for biostratigraphy than for palaeobotany studies precisely because, as xylomorphs, sporomorphs do not necessarily correspond to well identified botanic taxa.
- (2) The unusual occurrence of well preserved plant macrofossils. Usable plant macrofossils for palaeoflora reconstruction are essentially found in restricted deposits of exceptional preservation (e.g. Lagerstätten formations). Furthermore, fossil wood assemblages also

seem to be controlled by sedimentary processes, which could bias the palaeoflora and palaeoclimatic record (Garcia et al., 1998).

Conversely, vascular plant biomarkers present the advantage of being directly linked to specific plant taxa and their analysis is routinely performed on whole rocks which have typically not been collected from exceptional deposits. However, paradoxically, as pointed out by Pancost and Boot (2004), there are few examples of palaeoclimatic reconstructions using plant biomarkers. This can be explained by our lack of knowledge of palaeochemotaxonomy. Indeed, in order to describe palaeoclimatic changes in terms of temperature, humidity and seasonality evolution, it is necessary to comprehensively and precisely assess palaeoflora changes.

Recently, some research has focussed on the palaeochemotaxonomy of the Coniferales order (Otto et al., 1997, 2002a,b; Otto and Simoneit, 2001) because their representatives are particularly rich in terpenoids. Unfortunately, investigations of other taxa are scarce. However, the few studies attempting to establish relationships between biomarker compositions and non-coniferales taxa, such as ferns and angiosperms, while encouraging, need to be continued (Giannasi and Niklas, 1981; Paull et al., 1998; Zodrow and Mastalerz, 2001; Nguyen Tu et al., 2003; Jacob et al., 2005; Pšenička et al., 2005). Therefore, the catalogue of biomarkers having a palaeochemotaxonomic interest needs to be significantly expanded.

Until now, palaeochemotaxonomic data have been provided by the analysis of extant and fossil plants (including resin and amber). The main problems concerning the analysis of extant plants are:

- (1) Most of these studies have focussed on biological compounds which are either not of geochemical interest (such as nucleic acids, proteins, flavonoids, etc.) or which lose their chemotaxonomic value during early diagenesis (such as unsaturated fatty acids, glycerides, lignanoids, etc.).
- (2) Extrapolation of chemotaxonomic data to palaeochemotaxonomy is done assuming that the diagenetic effects are simply partial loss of labile functionalities and aromatization or hydrogenation without structural change.
- (3) The molecular compositions of taxa which do not have current representatives (e.g. Bennettitales and Caytoniales, which became extinct

toward the end of the Cretaceous; Taylor and Taylor, 1993) cannot be determined by analysis of extant plants.

Conversely, the main problems concerning the molecular analysis of fossil plants are:

- (1) Well identified and non-epigenetized macro-fossil plants (i.e. with initial organic matter still preserved and not replaced by minerals like calcite and pyrite) are not common, especially for non-woody plants.
- (2) Very few fossil taxa have been investigated for their molecular composition, especially considering the wide palaeobiodiversity of vascular plants.

Consequently, it appears necessary to find and develop other complementary analytical methods in order to fill the gaps in our knowledge of palaeochemotaxonomy.

In this paper, we propose an alternative experimental method based on artificial maturation. Initially developed to study the maturation of kerogen, confined pyrolysis (Monthieux et al., 1985; Landais et al., 1989) was recently successfully applied to provide evidence for a chemical genetic relationship between fossil and extant organisms (Stankiewicz et al., 2000).

The aim of this study was to calibrate the parameters for confined pyrolysis in order to generate, from a plant sample, the widest range of molecular plant biomarkers classically found in the geosphere. The plant example chosen is a representative of the Pinaceae conifer family, for which the molecular composition of the fossil representatives is well known. We believed the calibration of this approach would be when the distribution of biomarkers formed was in accord with the palaeochemotaxonomy of the selected taxon.

2. Experimental procedure

The plant was *Abies pinsapo* (Pinaceae, conifer) obtained from the Montet Botanical Garden (Université Henry Poincaré, Vandoeuvre-les-Nancy, France). Twigs and needles were finely cut using a solvent-washed razor blade. The material was dried under vacuum for 24 h in a desiccator at 45 °C before being pulverised in an agate mortar. The powder was stored in a freezer to prevent degradation by bacteria and fungi.

Pyrolysis was carried out in closed reactors consisting of gold sealed tubes of 40 mm length, 10 mm i.d. and 0.5 mm thickness. The tubes were sealed at one end and filled either with dry plant powder alone or with a mixture of dry plant powder and metal hydride under an Ar atmosphere (purity 99.995%) to avoid the presence of oxygen. The metal hydride was LiAlH₄ (Fluka No. 62420, purity ≥ 97%, powder form) and was used to generate terpanes during pyrolysis. Indeed, LiAlH₄ is a powerful reducing agent classically used in organic chemistry. When it is heated to >200 °C, it undergoes thermal degradation as illustrated by the reaction:



The H₂ will easily convert esters, carboxylic acids, aldehydes and ketones to alcohols (Wiench et al., 2004). The dihydrogen is in a supercritical phase under the experimental conditions (280 °C and 700 bar) and will easily hydrogenate alkenes.

Gold tubes were arc welded at the top end while the bottom end and sample were kept under a cold N₂ flow. Sealed gold cells were loaded into autoclaves and the experiments were carried out at 700 bar for 24 h.

Five reactors were loaded, each at a different temperature (150, 200, 250, 280 and 300 °C) in order for the samples to reach various stages of conversion. Each gold tube was filled with 80 mg of plant powder without metal hydride. For experiments using metal hydride, each was filled with 80 mg of plant powder and 120 mg of LiAlH₄.

After pyrolysis, the soluble organic matter was extracted using an accelerated solvent extractor (ASE 200; Li et al., 1998). The tubes were opened and placed inside pre-washed steel cells of the ASE 200. Extraction was carried out at 100 bar and 80 °C using CH₂Cl₂ as solvent. Purge gas was N₂. The heating phase and the static extraction times were both 5 min. Two extraction cycles were performed to ensure that the lipid fraction was completely extracted. CH₂Cl₂ was removed using a Zymark TurboVap LV and the extract was left to dry overnight. An aliquot was fractionated using liquid chromatography. The hydrocarbon fraction was separated from the polar fraction using an alumina column with successive elution with CH₂Cl₂ and CH₃OH/CH₂Cl₂ (50/50 v/v). Hydrocarbons were fractionated to recover aliphatic and aromatic components as well as residual polar fractions on a silica column by successive elution with pentane,

pentane/CH₂Cl₂ (65/35 v/v) then CH₃OH/CH₂Cl₂ (50/50 v/v). Total and fractionated extracts were diluted in hexane (4 mg/ml for aliphatic fractions and 8 mg/ml for the others) before being analysed using an HP 5890 Series II gas chromatograph coupled to an HP 5971 mass spectrometer (GC–MS). Aliquots of the total extracts and the polar fractions were methylated using tetramethylammonium hydroxide (TMAH) before GC–MS injection. The capillary column used was a DB-5 J&W column (60 m × 0.25 mm i.d. with 0.1 μm film thickness). The temperature programme was 70–315 °C at 15 °C/min to 130 °C, then 3 °C/min, followed by an isothermal stage at 315 °C for 15 min. He was the carrier gas (1 ml/min). The MS was operated in the electron ionization (EI) mode at 70 eV ionization energy and mass spectra were scanned from 50 to 500 Da using a quadrupole detector. Data were acquired and processed using the Agilent ChemStation software. Compounds were identified by comparison of their mass spectra with published spectra and those from the Wiley275 database, or by interpretation of MS fragmentation patterns.

3. Choice of plant and molecular composition of extracts

In order to develop and calibrate the confined pyrolysis for experimental palaeochemotaxonomy

purposes, we chose to pyrolyse a representative of the Pinaceae (Pine family, conifers) because the terpenoid compositions of extant and fossil Pinaceae are both well documented via bio- and geochemistry studies (e.g. Otto and Wilde, 2001; Otto and Simoneit, 2001). All species of Pinaceae synthesize large amounts of diterpenoid acids of the abietane class. Among the abietanoic acids, the most commonly cited is abietic acid but many others can be synthesized by Pinaceae, such as dehydroabietic, neoabietic, palustric and levopimaric acids (Rezzi et al., 2005). The diagenetic pathway of abietanoic acids is well known (Laflamme and Hites, 1978; Wakeham et al., 1980; Simoneit, 1986; Otto and Simoneit, 2001, 2002; Marchand-Geneste and Carpy, 2003). Two main processes can occur during the diagenesis of abietanoic acids: aromatization, favoured by oxidizing conditions, and hydrogenation, favoured by reducing conditions. Aromatization leads to the synthesis of retene via the formation of intermediate compounds such as dehydroabietic acid, dehydroabietins (18- and 19-norabietane-8,11,13-trienes), simonellite and tetrahydroretene. Hydrogenation leads to the formation of norabietanes (such as fichtelite) and possibly of abietanes, both of which can have many isomers (Fig. 1).

In this study, we chose to focus on the degradation of abietanoic acids to retene, abietanes and norabietanes during artificial maturation to calibrate the

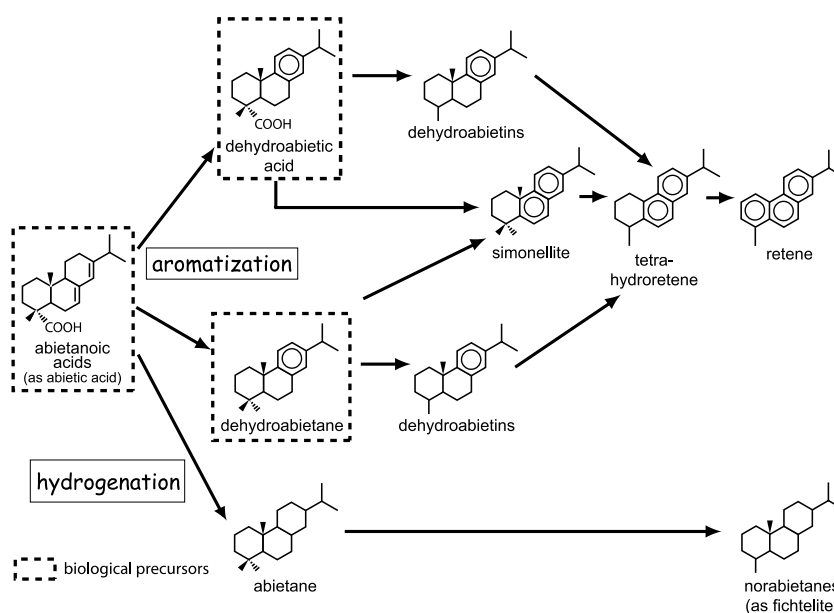


Fig. 1. Simplified diagenetic pathways of abietanoic acids. Two main pathways lead to the formation of either aromatic abietanes by defunctionalisation and dehydrogenation, or saturated diterpanes by defunctionalisation and hydrogenation.

pyrolysis parameters. *A. pinsapo* (Spanish fir) was chosen from the Pinaceae because previous studies of its molecular composition indicate that it is partic-

ularly rich in abietanoid compounds and that pimaranooids are absent (Barrero et al., 1992, 1993). Pimarane class bio-diterpenoids are usually wide-

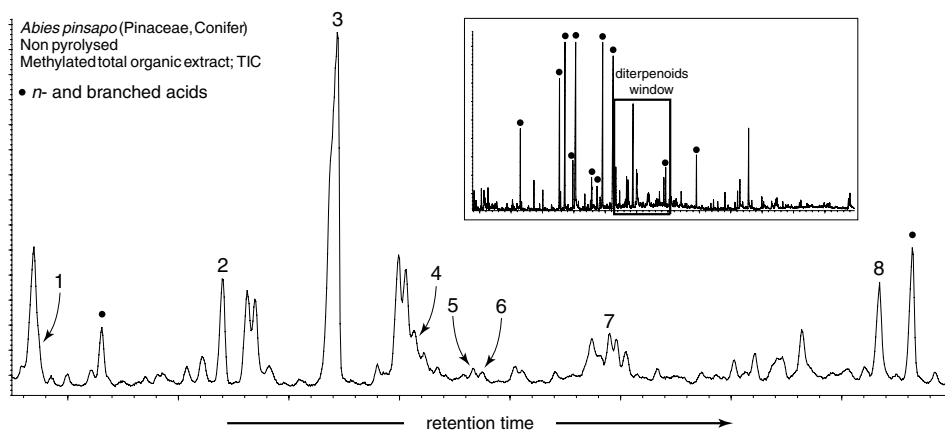


Fig. 2. Partial total ion chromatogram of methylated total extract showing diterpenoid content of fresh *Abies pinsapo*. Mass spectra and assignment of compounds 1–8 are given in Fig. 3.

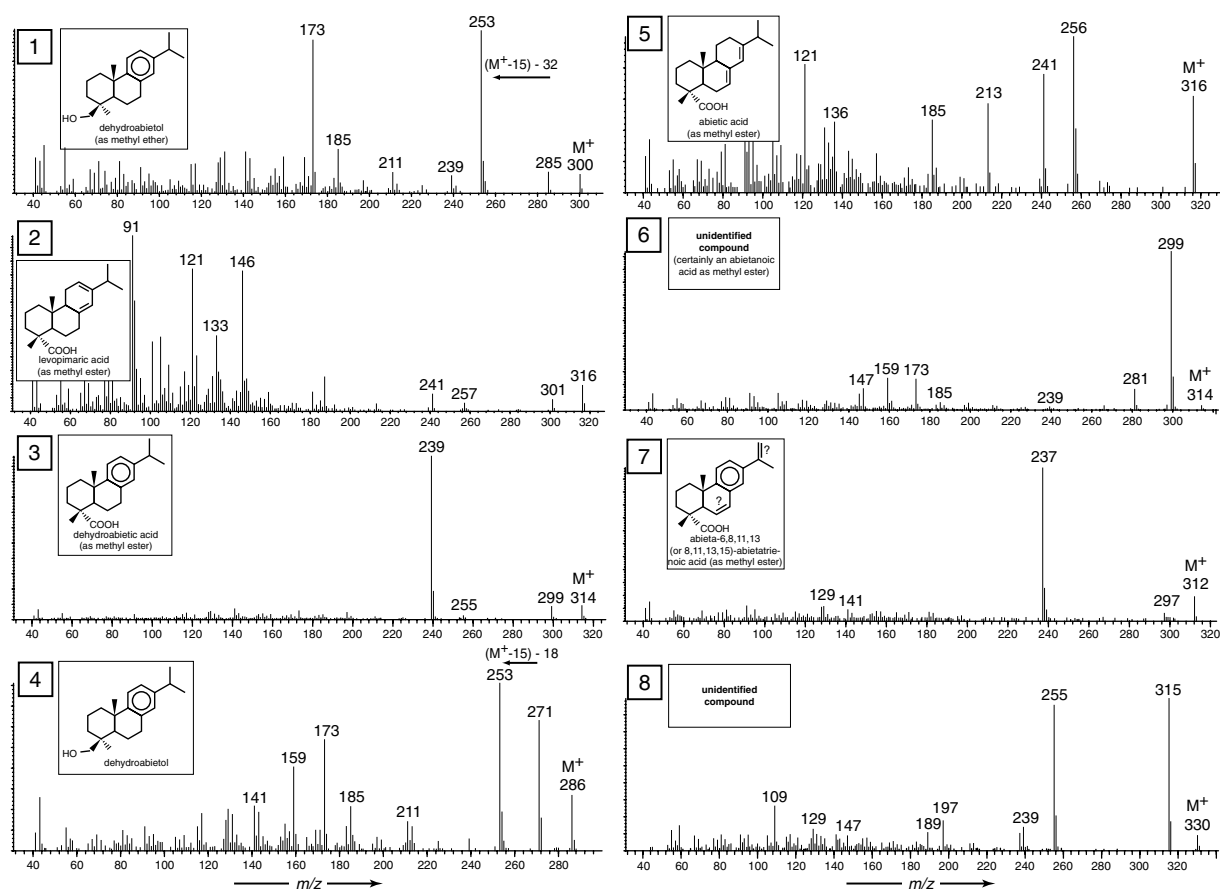


Fig. 3. Mass spectra and assignment of compounds 1–8 in Fig. 2.

spread in Pinaceae but their absence from *A. pinsapo* allowed us to focus on abietanes.

The molecular composition of the extract of fresh *A. pinsapo* obtained using GC–MS confirms the presence of many of these diterpenoids. Eight major abietane related products are present in the methylated total extract (1–8, Fig. 2). The mass spectra are shown in Fig. 3.

The spectrum of **1** shows M^{+} at m/z 300, which corresponds to a diterpenoid bearing one oxygen atom ($C_{20}H_{30}O$); the base peak at m/z 253 corresponds loss of CH_3OH from the $(M-15)^{+}$ ion at m/z 285. The compound is tentatively assigned as the methyl ether of dehydroabietol, which derives from the etherification of dehydroabietol during workup. Dehydroabietol itself is also tentatively identified in the extract as **4** by comparison of the spectrum with those in the Wiley275 database and the fragmentation of its silylated counterpart given by Otto and Simoneit (2001). Compound **2** is tentatively identified as the methyl ester of levopimaric acid because the spectrum matches very well with those in the Wiley275 database. By comparison with the spectrum in Simoneit (1977), **3** is tentatively identified as the methyl ester of dehydroabietic acid. Compound **5** is tentatively identified as the methyl ester of abietic acid because its spectrum matches very well with those in the Wiley275 database. Compound **6** is not clearly assigned but its fragmentation pattern suggests that it might be a methyl ester of an abietatrienoic acid, as indicated by the molecular ion at m/z 314 and the presence of m/z 159, 173, 185 fragments, which seem to be typical of abietatrienoic acids with the double bonds located in ring C and on the isopropyl group. Compound **7** has a spectrum similar to that of the methyl ester of dehydroabietic acid, but the fragments are lighter by 2 m/z units, which indicates the presence of a double bond. Generally, the double bond in the dehydroabietane structure is located at C-6 or C-15. Compound **7** could be abieta-6,8,11,13- or abieta-8,11,13,15-tetraenoic acid, both of which found in Pinaceae (Otto and Simoneit, 2001). Compound **8** remains unidentified. Dehydroabietane and a labdadienol are also present in lower amounts. The presence of abietanoids and labdanoids, together with the lack of pimaranooids, is consistent with the analysis of the extract from a fresh *A. pinsapo* by Barro et al. (1992, 1993). In the discussion below “acid(s)” refers to the methyl ester counterpart(s), since analysis was performed using a methylation agent (see above).

4. Results and discussion

4.1. Temperature calibration

First, the compounds eluting in the diterpenoid retention time window as presented in Fig. 2 were studied as a function of increasing pyrolysis temperature without $LiAlH_4$ (Fig. 4). At 150 °C, dehydroabietols (**1** and **4**) are present as well as abietic acid, together with unidentified abietanoic acids (**5** and **6**, respectively). The absence of levopimaric acid (**2**) may be attributed to its total conversion to dehydroabietic acid (**3**). Some *n*- and branched acids as well as unidentified compounds were newly formed or increased in relative intensity (like those eluting just after **6** for instance). Also, a large peak containing unidentified co-eluting compounds was formed. The unidentified compound **8** is absent. At 200 °C, *n*- and branched acids, dehydroabietols, abietic acid and dehydroabietic acid are still present. Many *n*- and branched acids and unidentified compounds occurring at 150 °C are now less abundant or absent. The distribution of the compounds eluting in the diterpenoid retention time window is similar from 250 °C to 300 °C. In this temperature range, dehydroabietic acid is the only abietanoic acid present and is more abundant than the *n*- and branched acids. Dehydroabietols can still be detected.

In a second step, the transformation of dehydroabietic acid into its diagenetic products was followed in order to determine the temperature at which the broadest distribution of abietanoic biomarkers is obtained. Indeed, all these compounds, from the less to the more diagenetically evolved, are widespread in the geosphere. With this aim, we studied the non-methylated total fractions obtained from pyrolysis performed without $LiAlH_4$, in order to avoid interference from *n*- and branched acids. Fig. 5 illustrates the relative abundance of the aromatic biomarkers involved in the diagenesis of dehydroabietic acid at the different pyrolysis temperatures (150, 200, 250, 280 and 300 °C). The degradation products of dehydroabietic acid obtained are dehydroabietins, simonellite, tetrahydroretene and retene, which were identified from the mass spectra published by Philp (1985).

At 150 °C, the distribution of abietane-class diterpenoids is dominated by dehydroabietic acid. The aromatic abietanoic hydrocarbons following this acid in the diagenetic pathway are below detection level.

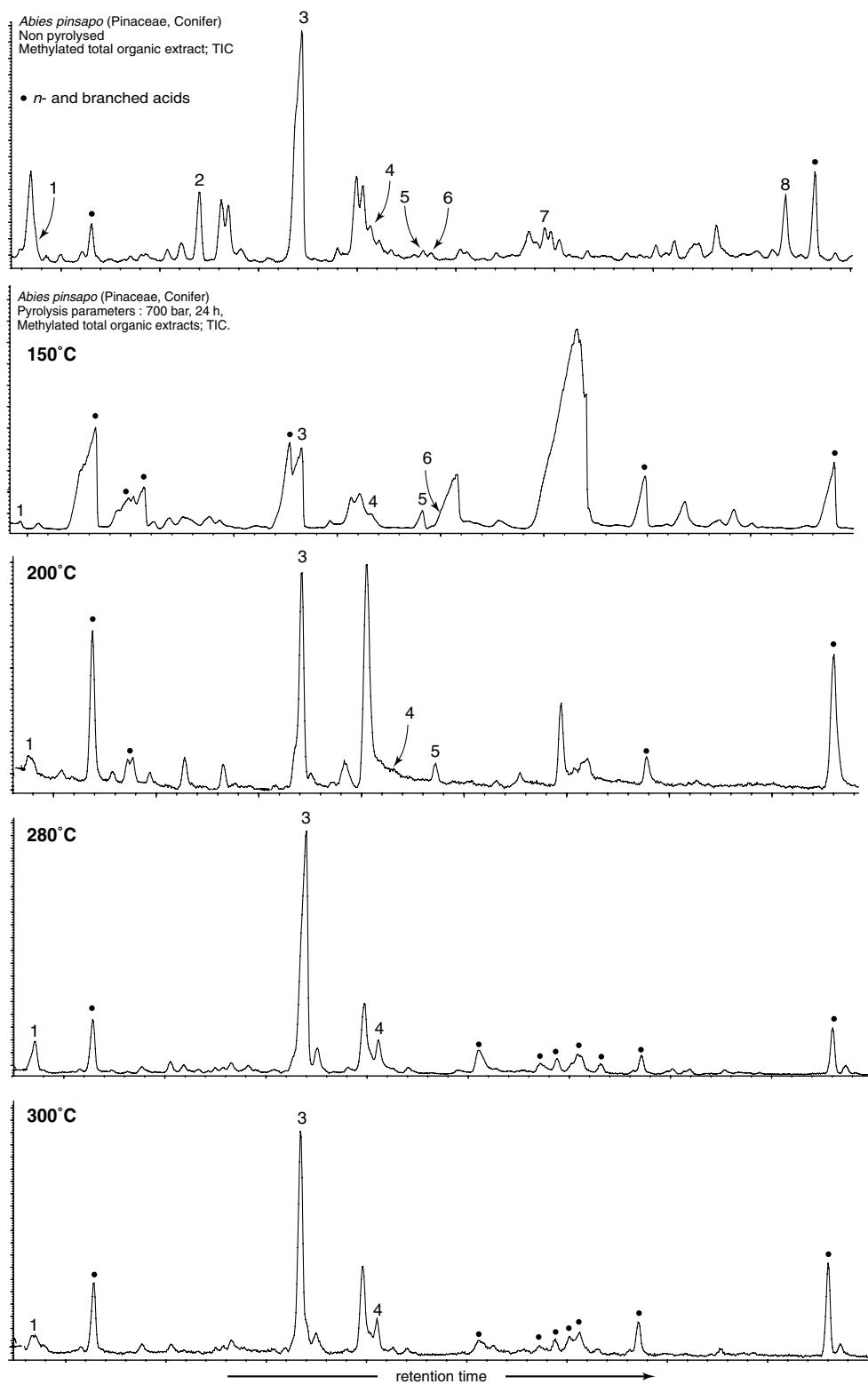


Fig. 4. Partial total ion chromatograms of methylated total extract showing diterpenoid composition of fresh and pyrolysed *Abies pinsapo*.

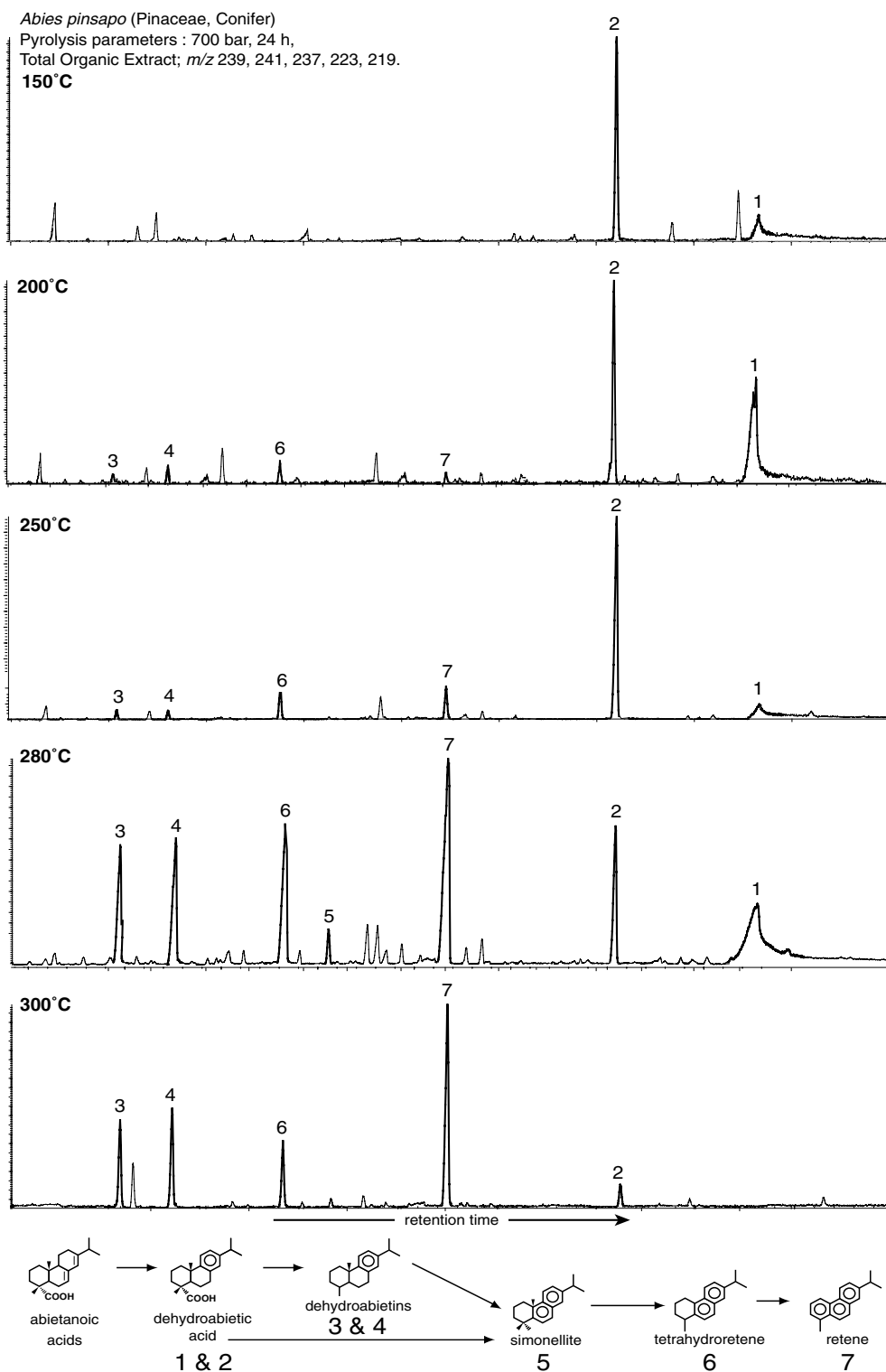


Fig. 5. Selected ion chromatograms of extract of *Abies pinsapo* pyrolysed at different temperatures (150, 200, 250, 280 and 300 °C). The selected ions are m/z 239 (diagnostic ion for dehydroabietic acid, peak 1, also present in methyl ester form, peak 2), m/z 241 (diagnostic for 19-norabieta-8,11,13-triene, peak 3, and 18-norabieta-8,11,13-triene, peak 4), m/z 237 (diagnostic for simonellite), m/z 223 (diagnostic for tetrahydroretene) and m/z 219 (diagnostic for retene).

At 200 °C, dehydroabietic acid is still very abundant and the relative proportions of dehydroabietins, tetrahydrotetene and retene are now measurable but are still in low abundance. Simonellite is still not detected.

At 250 °C, dehydroabietins, tetrahydrotetene and retene are present in higher relative proportions than at 200 °C but are still much less abundant than dehydroabietic acid. Simonellite remains undetected.

At 280 °C, dehydroabietins, tetrahydrotetene, retene and dehydroabietic acid have now similar abundances and are significantly above detection level. Simonellite is also present but in low relative proportion compared to the other abietanoids.

At 300 °C, the abundance of dehydroabietic acid decreases and retene is the most abundant abietanoid. The relative proportions of dehydroabietins, tetrahydrotetene and retene decrease compared to pyrolysis at 280 °C. Simonellite is now detected in low relative abundance.

Overall, the broadest distribution of abietanoic biomarkers is obtained at a pyrolysis temperature of 280 °C under 700 bar over 24 h. Lower pyrolysis temperatures do not lead to the formation of significant proportions of non-functionalised abietanoic biomarkers (dehydroabietins, tetrahydrotetene, simonellite and retene) while higher temperatures lead to the destruction of the functionalised biomarkers, which are often those having the greatest palaeochemotaxonomic value (Otto et al., 2002a).

The aliphatic fraction obtained at 280 °C was carefully analysed in order to determine if saturated abietanes and norabietanes were also formed. This fraction is composed of a large variety of unsaturated aliphatic compounds, including acyclic isoprenes such as phytene, *n*-alkenes and sterenes. A great number of peaks of very low intensity occurs in the diterpane retention time window and their mass spectra indicate that they are mainly mono-unsaturated diterpenes (Fig. 6). Most show a molecular ion at m/z 260, with an intense (M-15)⁺ fragment (m/z 245) suggesting that they are C₁₉H₃₂ tricyclic diterpenes. Spectrum **a** in Fig. 6 is similar to that described and tentatively identified by Hautevelle et al. (2006) as a norabietane monoene. Some peaks show spectra with few differences, which indicates the presence of various isomers and positions of the double bond. For example, spectrum **b** displays the same M⁺ and (M-15)⁺ fragments in similar relative abundances but differs via the lower intensities of the fragments at m/z 163, 189 and 217. The high intensity of the fragment at m/z 107 indicates that ring A bears two angular methyl groups (consistent with a norabietane structure) and a double bond. Other peaks show spectra having a molecular ion at m/z 274 and an intense (M-15)⁺ fragment (m/z 259) such as **c**, suggesting that they are C₂₀H₃₄ tricyclic diterpenes.

Unsaturated diterpenoids usually do not occur in the geosphere in such relative abundance and diver-

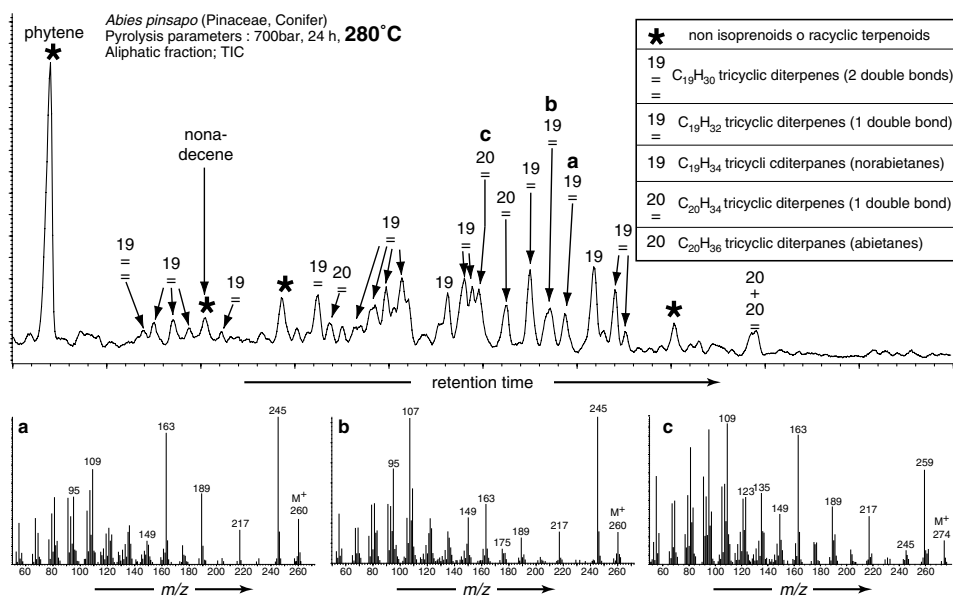


Fig. 6. Partial total ion chromatogram (diterpane retention time window) of aliphatic fraction of pyrolysis product at 280 °C showing high abundance of diterpenes and low abundance of diterpanes. Mass spectra of three of these diterpenes (**a**, **b** and **c**) are shown.

sity, although they have been reported in a few cases in very immature sediments and rocks (Venkatesan et al., 1986; Li and Johns, 1990; Hautevelle et al., 2006). Since diterpenoids also occur in saturated form in geological samples, pyrolysis with a reducing agent was carried out.

4.2. Artificial generation of diterpanes

Analysis of the aliphatic fraction of the pyrolysates obtained from pyrolysis using LiAlH_4 confirms that the diterpenes were hydrogenated (Fig. 7). Three main classes of compounds are represented in this fraction. Three peaks, labelled L,

have the same spectrum as that noted as **a** in Fig. 7. The base peak at m/z 123 and the molecular ion at m/z 278 suggest a $\text{C}_{20}\text{H}_{38}$ bicyclic diterpane. The compounds are assigned as labdane isomers by comparison of the spectra with those published by Noble et al. (1986). They remain in low abundance and are likely derived from the labdadienol, also detected in low abundance in the extract of fresh *A. pinsapo*. Eight peaks have a spectrum characterized by a base peak at m/z 109 and a molecular ion at m/z 262 (e.g. peak **b**). This indicates that they are $\text{C}_{19}\text{H}_{34}$ tricyclic diterpanes bearing two angular methyl groups on ring A. The peak noted **b** and the one eluting just in front of eicosane ($n\text{-C}_{20}$ alkane)

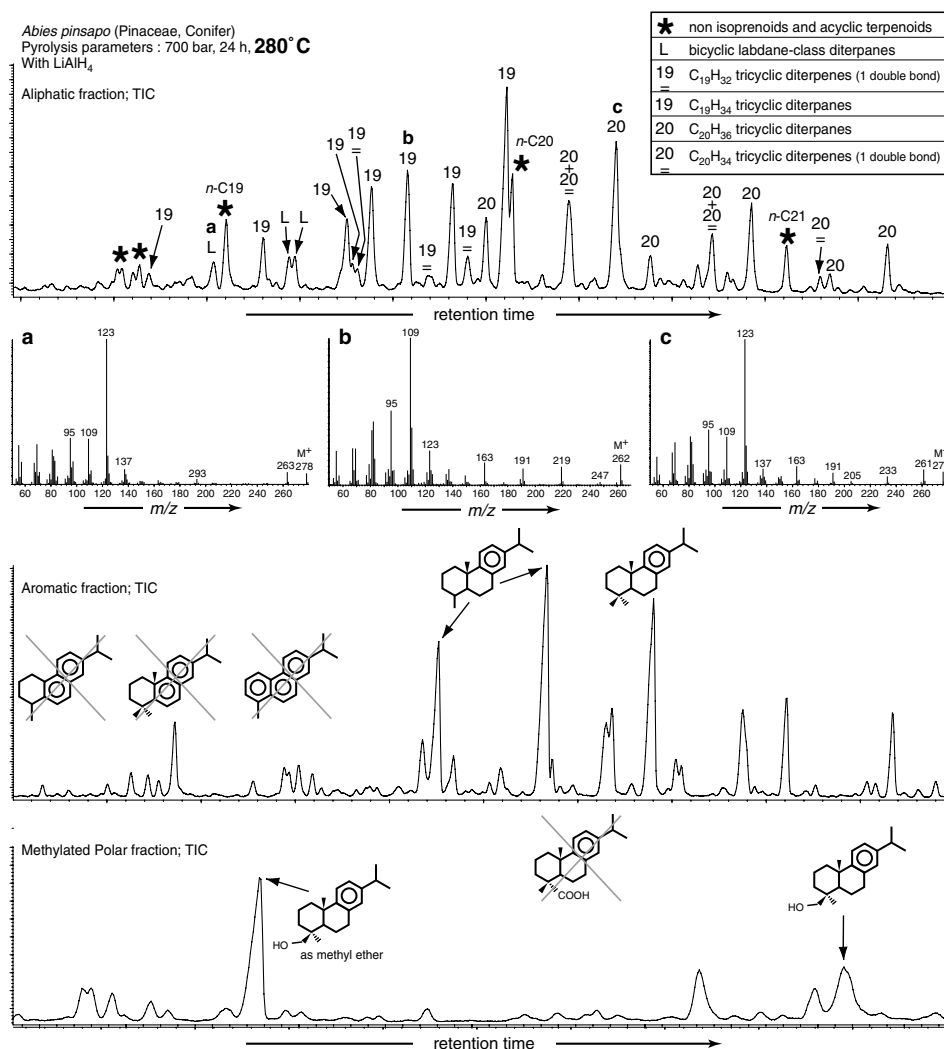


Fig. 7. Partial chromatograms (diterpane retention time window) of aliphatic, aromatic and polar fractions of pyrolysis product of *Abies pinsapo* at 280 °C with addition of metal hydride (LiAlH_4). Mass spectra of aliphatic compounds **a** (labdane), **b** (norabietane) and **c** (abietane), are shown for aliphatic fraction.

both show the spectrum noted **b** in Fig. 7. This is identical to the spectrum of a fichtelite reference published by Barrick and Hedges (1981). The other six peaks have almost similar spectra characterized by more intense fragments at m/z 163, 191 and 219. These spectra resemble those of the norabietane isomers also published by Barrick and Hedges (1981). These eight compounds could correspond to the eight possible epimers of norabietanes, considering two probable positions which can be occupied by the two methyl groups and the isopropyl group in the norabietane structure. Norabietane monoenes are still present in low relative abundance.

The compounds which elute mainly after eicosane display spectra with a base fragment at m/z 123 and a molecular ion at m/z 276, characteristic of the $C_{20}H_{36}$ tricyclic diterpanes. One of these spectra is noted **c** in Fig. 7 and corresponds to compound **b** bearing an additional methyl group on ring A. This compound is thus an abietane. Some of these peaks have spectra with fragments at m/z 163 and 191, which are a little more intense and correspond to abietane spectra published by Livsey et al. (1984) and Philp (1985). These compounds are thus assigned to other abietane isomers. Saturated pimaranes and norpimaranes can be easily distinguished from the abietanes and norabietanes by the intensity of the $(M-29)^+$ fragment and are not present in this fraction. This is consistent with the lack of pimarane precursors in the extract of fresh *A. pinsapo*.

In the aromatic fraction of *A. pinsapo* pyrolysed in the presence of $LiAlH_4$, the two isomers of dehydroabietin, as well as dehydroabietane, are present. However, abietanoids bearing more than one aromatic ring, such as simonellite, tetrahydroretene and retene, are absent. This indicates that the reducing conditions imposed by the thermolysis of the metal hydride inhibit aromatization. The other compounds eluting in the diterpenoid retention time window remain unidentified and their occurrence in geological samples has not, to our knowledge, been reported.

The polar fraction shows a strong peak corresponding to dehydroabietol in the diterpenoid window. Dehydroabietic acid is below the detection limit. This is attributed to the effect of $LiAlH_4$, which converts carboxylic acids to alcohols. The dehydroabietic acid was therefore certainly converted to dehydroabietol.

All aliphatic compounds of interest produced by the pyrolysis with $LiAlH_4$ are found in ancient sediments.

4.3. Predictive “palaeochemotaxonomy” of *A. pinsapo*

The results clearly demonstrate that:

- (1) Pyrolysis without metal hydride produces aromatic and polar biomarkers which are typically detected in sediments and sedimentary rocks. Yet, such experimental conditions are unable to generate saturated plant biomarkers.
- (2) Pyrolysis using metal hydride mixed with the plant sample produces saturated plant biomarkers such as abietanes and norabietanes, but is unable to generate their aromatic counterparts. Moreover, pyrolysis with the metal hydride converts carboxylic acid, aldehyde and ketone biological precursors to alcohol biomarkers, which could considerably bias palaeochemotaxonomic interpretation.

Therefore, the molecular composition of a “virtual fossil” of *A. pinsapo* can be inferred from the aliphatic fraction of the pyrolysate obtained at 280 °C, 700 bar for 24 h using $LiAlH_4$ and the aromatic and polar fractions of the pyrolysate obtained at 280 °C, 700 bar for 24 h without the use of $LiAlH_4$. Their whole chromatograms are represented in Fig. 8.

In addition to the diterpanes described above, the aliphatic fraction of the *A. pinsapo* pyrolysed with $LiAlH_4$ contains:

- *n*-alkanes;
- $C_{15}H_{28}$ sesquiterpanes (M^+ : m/z 208) with mass spectra matching those of muurolanes and amorphanes from the Wiley275 database;
- C_{29} steranes and minor amounts of C_{28} steranes derived from their alcohol and ketone precursors, which are typical of terrestrial plants (Volkman, 1986).

The aromatic fraction of *A. pinsapo* pyrolysed without $LiAlH_4$ contains:

- a dimethylnaphthalene which was not precisely identified;
- 2,3-dihydro-1,1,4,5,6-pentamethylindene noted as **3** in Fig. 8;
- aromatic sesquiterpenoids such as dihydro-*ar*-curcumene (**1**), cuparene (**2**), calamene (**4**), cadinatriene (**5**) and cadalene (**6**). Dihydro-*ar*-curcumene

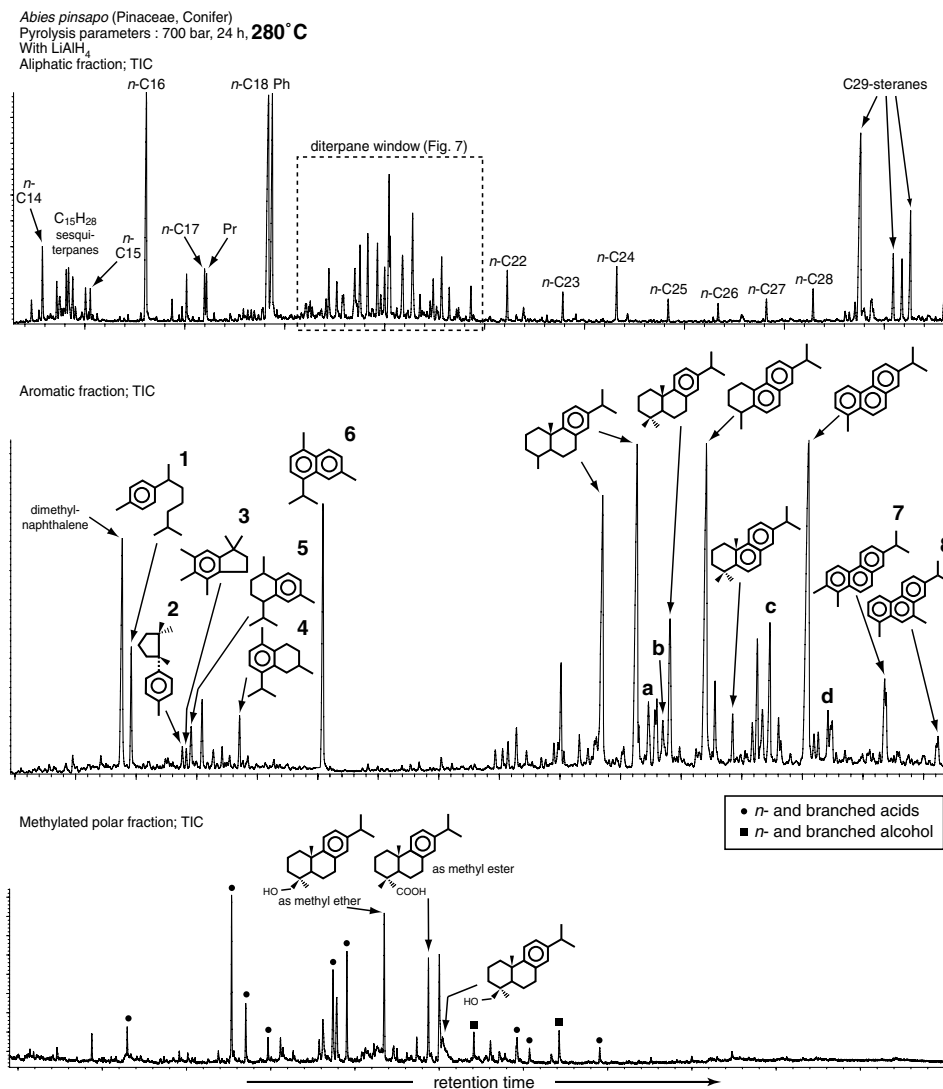


Fig. 8. Total ion chromatograms of aliphatic (pyrolysis with LiAlH₄), aromatic and polar fractions (both pyrolysis without LiAlH₄) of pyrolysates of *Abies pinsapo* at 280 °C. *n*-C#: *n*-alkanes with # carbon atoms; 1: dihydro-*ar*-curcumene (identified from spectrum in Ellis et al., 1995); 2: cuparene (identified from spectrum in Grantham and Douglas, 1980); 3: 2,3-dihydro-1,1,4,5,6-pentamethylindene (identified using Wiley275 database); 4: calamenene (identified from spectrum in Simoneit and Mazurek, 1982); 5: cadina-1(10), 6,8-triene (identified from spectrum in Simoneit and Mazurek, 1982); 6: cadalene (identified from spectrum in Philp, 1985); 7: 2-methylretene (identified from spectrum in Bastow et al., 2001); 8: 9-methylretene (identified from spectrum in Alexander et al., 1995). Spectra of compounds **a**, **b**, **c** and **d** are shown in Fig. 9.

certainly derives from sesquiterpenoids related to juvabione identified in the extract of fresh *A. pinsapo* by Barrero et al. (1989);

- 2- and 9- methylretenes (respectively, peaks 7 and 8 in Fig. 8) in addition to the aromatic diterpenoids of the abietane class described above;
- several compounds which have, to our knowledge, never been reported. They are in lower abundance than the abietanes and spectra of four of them, noted from **a** to **d**, are represented in Fig. 9. Com-

pounds **a** and **b** were tentatively identified as diaromatic abietanes with an open naphthenic ring A. These compounds are believed to be, respectively, 3-ethyl-4-butyl-8-isopropyl-naphthalene and 3-methyl-4-pentyl-8-isopropyl-naphthalene. A similar type of abietane structure with an open ring has been reported by Ellis et al. (1996).

The spectrum of compound **c** displays M⁺ at *m/z* 238, corresponding to C₁₈H₂₂. The loss of 43 mass

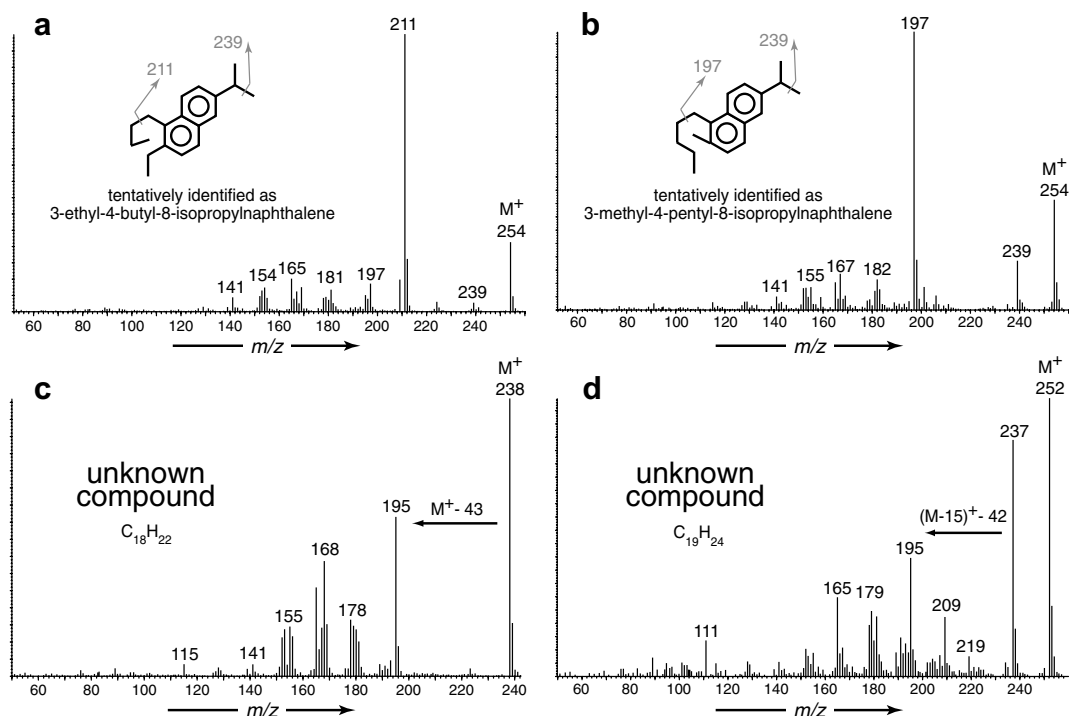


Fig. 9. Mass spectra of peaks **a**, **b**, **c** and **d** in Fig. 8 and suggested mass spectral cleavage patterns for tentative identification of biomarkers **a** and **b** (respectively, 3-ethyl-4-butyl-8-isopropyl-naphthalene and 3-methyl-4-pentyl-8-isopropyl-naphthalene).

units indicates that it probably bears a propyl side chain or an isopropyl group. It has been found in relatively high abundance in extracts of Callovo-Oxfordian claystones from the Paris basin and might possibly have important palaeochemotaxonomic value (Hautevelle, 2006).

The spectrum of compound **d** displays a base peak as molecular ion at m/z 252. The $(M-15)^+$ fragment is relatively intense as are, to a lesser extent, fragments at m/z 165, 179 and 195. This fragmentation pattern seems characteristic of a $C_{19}H_{24}$ diaromatic tricyclic diterpenoid with 2 methyl groups at C-4, such as simonellite, diaromatic totarane and diaromatic sempervirane for which spectra have been published by Philp (1985), Otto et al. (1997) and Tuo and Philp (2005), respectively. Unfortunately, the structural class of this unidentified component is unclear, although it could have important palaeochemotaxonomic value if found in ancient sediments.

The polar fraction of *A. pinsapo* pyrolysed without $LiAlH_4$ and analysed using TMAH as methylation agent contains:

- C_{29} sterones and sterols typical of terrestrial plants (Volkman, 1986);

- several linear and branched carboxylic acids, alcohols and ketones;
- polar abietanoids reported in fresh *A. pinsapo* (dehydroabietol and dehydroabietic acid);
- some compounds which remain unidentified.

The study of the molecular content of the above three fractions (i.e. aliphatic fraction obtained using $LiAlH_4$, aromatic and polar fractions obtained without the use of $LiAlH_4$) thus makes it possible to predict the possible terpenoid composition of a “virtual *A. pinsapo* fossil”. The consistency of the prediction is verified by comparison with the components of fossil Pinaceae as described in published palaeochemotaxonomic studies. The three fractions all contain both generic plant biomarkers like the cadinane-class sesquiterpenoids (even if these biomarkers seem to be more frequent in conifers than other land plants) and more specific biomarkers. The muurolanes and labdanes are semi-specific biomarkers of conifers and occur in almost all conifer families except Taxaceae. The curcumanes and amorphanes found in our pyrolysates are also specific for conifers and are essentially produced by Pinaceae and Cupressaceae (Otto and Wilde, 2001).

The high content in abietanes is also characteristic of conifers and a detailed study of their distribution allows characterization at the family level. Indeed, the presence of abietanoic acids and dehydroabietol, coupled to the lack of ketophenolic and phenolic abietanes, is typical of fossil Pinaceae. Furthermore, the absence of totaranes and tetracyclic diterpenoids (kauranes, phyllocladanes, beyeranes, etc.) is another specificity of the fossil Pinaceae (Otto et al., 1997; Otto and Simoneit, 2001). Cuparene, in theory a specific biomarker for one single conifer family, the Cupressaceae *s. st.*, (Grantham and Douglas, 1980; Otto and Wilde, 2001; Otto et al., 2002b) was found in low abundance within the aromatic fraction of the pyrolysed *A. pinsapo*.

Therefore, the distribution of the plant biomarkers experimentally synthesized by confined pyrolysis of *A. pinsapo* using the procedure developed herein matches well with those described in conventional palaeochemotaxonomic studies of Pinaceae (e.g. Heppenheimer et al., 1992; Staccioli et al., 1999, 2000; Otto and Simoneit, 2001, 2002). This experiment also suggests that a low proportion of cuparene is not necessarily a strict indicator of the Cupressaceae family, even if it is generally believed that this biomarker is very specific for Cupressaceae.

Of course, a fossil *A. pinsapo* could actually display a slightly different molecular composition as a result of its sedimentological and diagenetic history. Indeed, the maturation state as well as the biological, physical and chemical characteristics of the depositional and interstitial environments can have a major impact on the molecular composition during diagenesis. It could notably affect the functionalised molecule vs. hydrocarbon ratio and the aromatic vs. aliphatic biomarker ratios as well as possible structural configurations. However, the biomarker distribution obtained with our experimental procedure is believed to represent an average composition, including functionalised, aromatic and aliphatic biomarkers.

5. Conclusions

Confined pyrolysis of a Pinaceae species (*A. pinsapo*) at 700 bar, 280 °C for 24 h, with and without addition of LiAlH₄ to the plant powder, allowed laboratory synthesis of a set of plant biomarkers commonly found in geological samples. The distribution of these biomarkers matches very well those

described in conventional palaeochemotaxonomic studies of Pinaceae.

The biomarkers produced also match what is expected from the preservation pathway of conifer-derived terpenoids. Reproduction of the experiment using not only other vascular plants, but also other living organisms (bacteria, algae, and animals), for which fossil molecular signatures are unknown, should provide new and useful palaeochemotaxonomic data.

In the long term, these data should allow:

- finding palaeochemotaxonomic trends for non-investigated taxa;
- making more specific and possibly correcting trends already evidenced in previous conventional palaeochemotaxonomic studies;
- highlighting the limitations of these chemical trends, as pointed out for instance in this study by the presence of a specific biomarker for Cupressaceae in the Pinaceae pyrolysis products;
- proposing diagenetic pathways for biogenic compounds which have been less studied than those of abietic acid.

This information is essential for suitably interpreting the distribution of vascular plant biomarkers in terms of palaeoflora and possibly palaeoclimate. The data could also be useful for organic archaeological chemistry. Indeed, plant biomarkers are frequently found in material of archaeological interest (coatings, varnishes, paints, pitches, manufactured oils, desiccated botanical remains, mummy balms, etc.; e.g. Buckley et al., 2004; Colombini et al., 2005) and could be used for assessing their origin and manufacturing process.

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