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Terpenoids in extracts of Lower Cretaceous ambers from the Basque-Cantabrian Basin (El Soplao, Cantabria, Spain): Paleochemotaxonomic aspects

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ABSTRACT

The composition of terpenoids from well preserved Cretaceous fossil resins and plant tissues from the amber bearing deposits of El Soplao and Reocín in Cantabria (northern Spain) have been analyzed using gas chromatography–mass spectrometry and the results are discussed using the terpenoid composition of extant conifers as a reference. Amber is present at many horizons within two units of coastal to shallow marine siliciclastics of Albian and Cenomanian age. The fossil resins are associated with black amber (jet) and abundant, well preserved plant cuticle compressions, especially those of the extinct conifer genus *Frenelopsis* (Cheirolepidiaceae).

We report the molecular characterization of two types of amber with different botanical origins. One of them is characterized by the significant presence of phenolic terpenoids (ferruginol, totarol and hinokiol) and pimaric/isopimaric acids, as well as their diagenetic products. The presence of phenolic diterpenoids together with the lack of abietic and dehydroabietic acids excludes both Pinaceae and Araucariaceae as sources for this type of amber. The biological diterpenoid composition is similar to that observed for extant Cupressaceae. The second type of amber is characterized by the absence of phenolic terpenoids and other specific biomarkers. Some terpenoids with uncertain structure were detected, as well as the azulene derivative guaiazulene. Our results suggest that the amber from Cantabria could be fossilized resin from *Frenelopsis* and other undetermined botanical sources. The biological terpenoid assemblage confirms a chemosystematic relationship between *Frenelopsis* and modern Cupressaceae.

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1. Introduction

Amber is fossilized resin produced from the exudates of conifers and certain angiosperms and is considered to be one of the few fossil deposits of exceptional preservation (*Konservat Lagerstätten*), because it permits the conservation of fossil organisms with all their delicate anatomical details. Fossil resins not only preserve the anatomy of fossil life forms that were trapped as biological inclusions, but also constitute a valuable source of information about their own botanical origin, ancient terrigenous ecosystems and climatic change by means of their chemical composition (Anderson and Crelling, 1995).

Analysis of the chemical composition of fossil resins is not straightforward, because the original biochemical fingerprints of the resins are usually modified during diagenesis, with the bioterpenoids (unmodified biosynthetic natural products) being transformed into geoterpenoids (diagenetic products of degraded bioterpenoids that are found in amber and fossil plant tissues; Otto et al., 2007). Despite these diagenetic alterations, geoterpenoids retain the basic skeletal structures of their biological precursors and can be used as molecular markers (biomarkers; Peters et al., 2005; Marynowski et al., 2007). Conifers synthesize mainly diterpenoids, which are, along with sesquiterpenoids, the compounds that provide the best results as diagnostic biomarkers of conifers and their resins (Otto and Wilde, 2001). Among the diterpenoids preserved in amber, labdane derivatives and non-phenolic abietane diagenetic derivatives have the most limited chemotaxonomic value, as they occur in all conifer families. On the other hand, phenolic terpenoids, such as ferruginol and totarol, are produced only by

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the members of the families Cupressaceae and Podocarpaceae (Cox et al., 2007). Therefore, the chemotaxonomic value of these compounds is very high and their presence in amber provides very useful palaeobotanical information. Although the preservation potential of polar biomarkers is considered to be low (Otto et al., 2007), the oldest polar diterpenoids have been identified in extracts of Middle Jurassic fossil conifer wood from Poland (Marynowski et al., 2007), and diterpenoid derivatives could also be liberated from a Carboniferous amber by pyrolysis (Bray and Anderson, 2009).

Recently, a new Cretaceous amber deposit with exquisite, well preserved fossil organisms, mostly insects, has been discovered in northern Spain (Rábago village in El Soplao territory, Cantabria; Menor-Salván et al., 2009; Najarro et al., 2009). Based on preliminary infrared spectroscopy of the El Soplao amber (Najarro et al., 2009) and previous gas chromatography–mass spectrometry (GC–MS) studies on amber from a neighboring site in Álava (Alonso et al., 2000; Chaler and Grimalt, 2005), it has been suggested that exudate from *Agathis* (a conifer of the family Araucariaceae) was the most likely source of this amber, as has also been proposed for other Cretaceous ambers (e.g. Lambert et al., 1996; Alonso et al., 2000; Poinar et al., 2004; Chaler and Grimalt, 2005; Delclòs et al., 2007). This speculation was largely based on the presence of some geoterpenoids that may have been derived from agathic and pimic acids. However, although those compounds and their diagenetic derivatives are characteristic of Araucariaceae, they are not diagnostic, because they can also be found in other extant conifer families (Otto et al., 2007). Moreover, Alonso et al. (2000) have reported the presence of the phenolic abietane ferruginol in the Álava amber samples, indicating that more extensive study of the chemotaxonomic information contained in the amber is necessary to establish its definite botanical origin.

In addition, meso- and macrofossil plant remains of Araucariaceae are absent in these amber bearing deposits, although there are plenty of cuticles and remains of other vascular plants, especially the genera *Frenelopsis* sp. and *Mirovia* sp., of the extinct conifer families Cheirolepidiaceae and Miroviaceae, respectively (Gomez et al., 2002a; Najarro et al., 2009). This is also the case in many other amber deposits from the Cretaceous of Spain and France (e.g. Delclòs et al., 2007; Néraudeau et al., 2008). Thus, the recurrent association of amber with cuticles of Cheirolepidiaceae and Miroviaceae, along with the lack of Araucariaceae remains (except for a small amount of pollen grains in the sediments) (Barrón et al., 2001), challenges the proposed origin of the amber. Since chemical evidence has not yet given a definitive answer, more convincing proof is necessary to accept Araucariaceae as the source of the resin.

In this study, amber pieces and associated fossil leaves from the Cretaceous amber bearing deposit of El Soplao (Cantabria; Fig. 1) were systematically analyzed using complementary techniques such as infrared spectroscopy (FTIR) and GC–MS. The overall aim was to identify the terpenoids preserved and their diagenetic transformation products in the fossil resin and to determine their possible botanical sources. Due to exceptional preservation, the amber bearing deposit at El Soplao offers a unique opportunity to compare the molecular composition of the amber with that of plant remains from the family Cheirolepidiaceae and Miroviaceae, which appear in the same deposit. A morphological similarity between extinct Cheirolepidiaceae and extant Cupressaceae has been described, but their relationship remains speculative due mainly to the lack of molecular evidence (Broutin and Pons, 1975; Alvin and Hluštík, 1979; Seoane, 1998; Miller, 1999; Farjon, 2008). As Cheirolepidiaceae is an extinct family, the connection between the two families could aid in the chemotaxonomical study of amber and in the confirmation of the botanical origin. We present data of two separate types of amber found in the El Soplao deposit and discuss their botanical origin using comparative chemotaxonomy

based on modern resin compositions and related terpenoids found in amber associated fossils.

2. Samples and methods

2.1. Geological background

The analyzed samples belong to the Cretaceous succession at the northwestern margin of the Basque-Cantabria Basin in northern Spain. During the Cretaceous, the evolution of this basin was controlled by extensional, and perhaps strike-slip, deformation associated with the opening of the North Atlantic Ocean and the Bay of Biscay (e.g. Le Pichon and Sibuet, 1971; Rat, 1988; García-Mondéjar et al., 1996; Soto et al., 2007). Rifting during the Late Jurassic–Early Cretaceous led to the formation of several narrow sub-basins controlled by E–W, NW–SE and SW–NW trending faults; these basins host both continental and marine sediments of variable thickness (García-Mondéjar et al., 1996; Soto et al., 2007).

The study area lies in the Cantabria region immediately to the north of the Cabuérniga Ridge (Fig. S1; supplementary material), an E–W fault zone that represents a Late-Variscan structure reactivated first as a paleo-high bounded by normal faults during the Early Cretaceous, and later as reversal faults during the widespread Cenozoic (Pyrenean) compression. The Lower–Middle Cretaceous (Barremian–Early Cenomanian) deposits in the study area are weakly deformed and affected only by gentle folding. They are composed of a relatively thin (~200–800 m) syn-rift sequence that lies unconformably on Carboniferous to Lower Jurassic basement (Fig. S1).

A simplified synthesis of the stratigraphy in the El Soplao and Reocín areas is shown in Fig. 1, with formation names according to Hines (1985) and revised by Najarro et al. (2009). The amber bearing deposit at El Soplao is included within the Las Peñas Formation (Fig. 1), a Lower Albian unit (~112–110 Ma) of continental to transitional marine siliciclastics. Detailed descriptions of field sections, depositional environments and fossil content of this unit are given in Najarro et al. (2009). Within the outcrop, the El Soplao amber deposit is characterized by about 1.5–2 m of dark, carbonaceous lutites, siltstones and sandstones with interbedded, centimeter to decimeter layers with remarkable accumulations of plant remains and amber pieces of different sizes and forms (Fig. 2A and B). Most amber pieces show a blue-purple color under normal sunlight and bright milky blue fluorescence under ultraviolet light. Plant cuticles are very abundant in the levels associated with amber (Fig. 2C). They are mainly assigned to the conifer genera *Frenelopsis* and *Mirovia*, along with other more occasional leaves of the ginkgoalean genera *Nehvizdya* and *Pseudotorellia* (Najarro et al., 2009). In most of the amber beds, leaves of the genus *Frenelopsis* of the extinct conifer family Cheirolepidiaceae are the dominant macro-botanical remains. *Frenelopsis* were xeromorphic plants adapted to coastal habitats and probably grew mainly in brackish coastal marshes and mangroves, but were adapted to a wider range of habitats (Gomez et al., 2002a, 2003).

The amber deposit at Reocín (Fig. S1; supplementary material) is slightly younger than the El Soplao amber deposit. It is included within the Bierva Formation (Fig. 1), a Latest Albian–Early Cenomanian (~102–99 Ma) unit composed of about 250 m of tidal dominated, estuarine siliciclastic deposits in the study area (Hines, 1985). Within this unit, the amber accumulations are associated with carbonaceous claystones and tidal channel sandstones developed in estuarine mouth subtidal areas (López-Horgue et al., 2001). Despite the differences in age, the Reocín amber shows the same composition as the El Soplao amber. Thermal maturity indicators (vitrinite reflectance) of macerals in the El Soplao deposit reveal minor changes in the organic matter of the resins during their diagenetic history and maximum thermal conditions during burial of

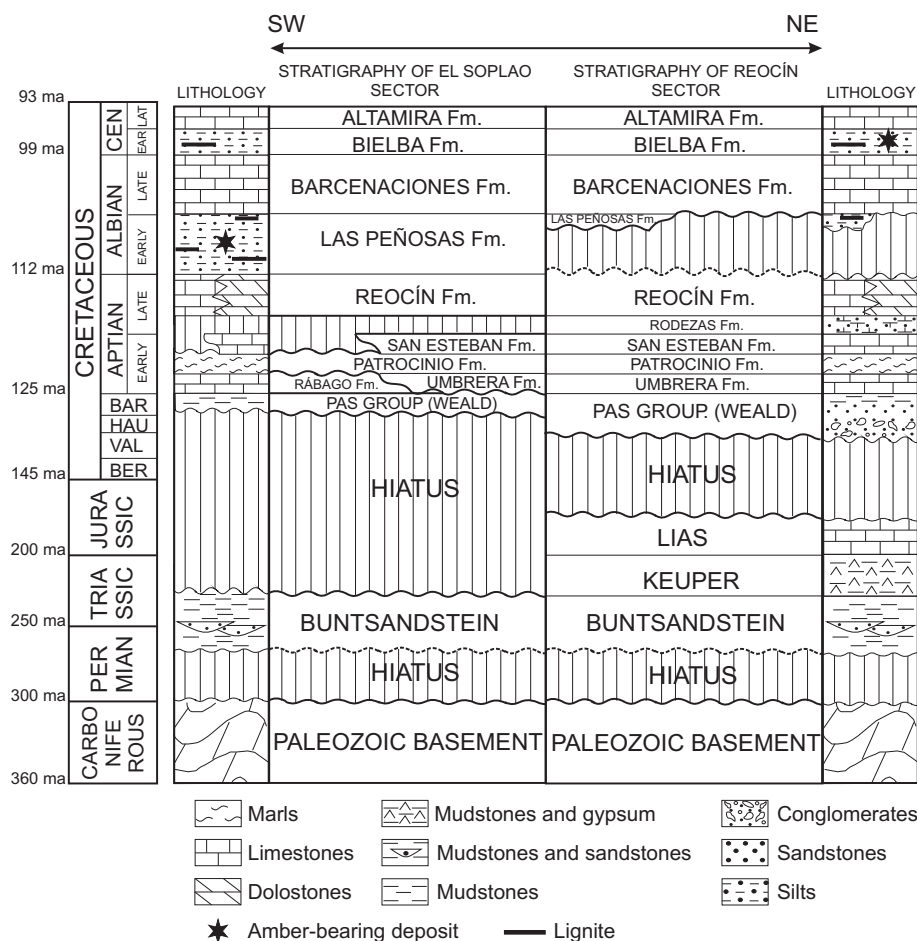


Fig. 1. Chrono- and lithostratigraphy of the El Soplao and Reocın areas (modified from Hines, 1985). Chronostratigraphy after Gradstein et al. (2004).

~60–70 °C (Supplementary material). Consequently and due to its higher transparency and lack of inclusions and interferences, the El Soplao amber was used preferentially for the chemosystematic study.

2.2. Sampling

Amber pieces, jet (black amber), fossil wood and sediments rich in plant cuticles were collected from the El Soplao deposit during a recent excavation in October 2008. Two types of amber pieces were found at the deposit in the same sedimentological and taphonomical context: A type, characterized by a strong blue-purple color under natural light, purple-reddish under artificial light and less abundant B type, yellow-honey under artificial light and honey with a bluish tinge under natural light. We collected the two types of amber present and the black amber associated with amber of type A and fossil plant tissue. Plant cuticles were obtained from claystones by rinsing the plant rich sediment in an ultrasonic bath of distilled water to remove all the clay and silt sediment. The organic residue (Fig. 2C) was air dried. Plant fragments and leaves from different families were distinguished and separated under a stereomicroscope.

2.3. Analytical methods

2.3.1. Infrared spectroscopy (FTIR)

IR spectra of pulverized solid amber were obtained using a Nexus Nicolet FTIR spectrometer in the 4000–400 cm^{-1} range.

2.3.2. Extraction and fractionation

For the analytical characterization, two representative single pieces (A and B; Table 1) of amber of about 50 g, with the highest transparency available and free of major inclusions, crusts and debris, were collected from the El Soplao deposit. Each piece was crushed and extracted for 4 h with dichloromethane:methanol (2:1 v:v) using a Buchi model B-811 automatic extractor. The extractable material constitutes 16% of the total amber weight on average. One aliquot of extract was injected directly into the port of the gas chromatograph.

The bulk extract was then processed in order to purify the phenolic terpenoid fraction and the acidic fraction and to identify unambiguously the minor components with higher chemosystematic value. The aim was to establish a complete descriptive composition of the amber sample. The extract was concentrated to a volume of 20 ml and fractionated by flash chromatography on silica gel. The elution was performed using *n*-hexane, dichloromethane, dichloromethane:methanol (1:1 v:v), and methanol as eluents and 25 fractions of 1.5 ml were collected using an automatic fraction collector. Each fraction was concentrated by evaporation of the solvent under N_2 and analyzed by GC–MS. The fractions with similar compositions were combined. The polar fraction (eluted with methanol) and the fractions containing ferruginol were recombined, further separated using a glass column (20 cm) filled with chromatographic grade silica gel, and eluted sequentially with *n*-hexane:dichloromethane (1:1 v:v), pure dichloromethane, dichloromethane:methanol (1:1 v:v) and methanol. Four fractions of 20 ml were collected, designated A to D. All fractions were dried and the alcohols and acids converted to trimethylsilyl derivatives

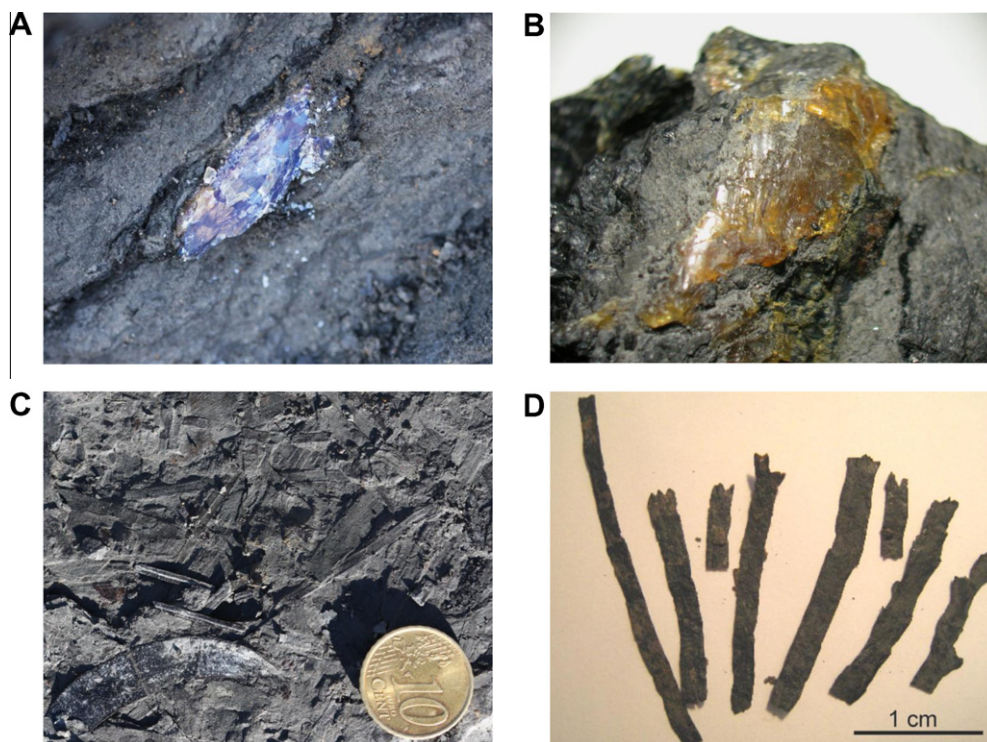


Fig. 2. Photographs of amber and palaeobotanical components used in this study. (A) In situ amber piece of type A (6 cm) from the El Soplao deposit, showing blue-purple color under sunlight. (B) Amber piece of type B (3 cm) on fossil wood under sunlight. (C) In situ sediments showing their palaeobotanical components: fossil leaves of *Mirovia* and *Frenelopsis* and one leaf of Ginkgoaceae. (D) Selected leaves of *Frenelopsis* sp. were used for biomarker analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by reaction with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 65 °C for a period of 3 h. Finally, the derivatized fractions were diluted with *n*-hexane and injected into the port of the gas chromatograph.

To study the molecular content of fossil *Frenelopsis* and *Mirovia* leaves (Fig. 2), 5 g of leaves were extracted for 4 h with dichloromethane:methanol (2:1 v:v) using a Büchi model B-811 automated extractor. The bulk extract was filtered and analyzed directly by GC–MS. The extract was then fractionated using silica gel chromatography in two fractions by elution with *n*-hexane:dichloromethane (3:1 v:v) and dichloromethane:methanol (4:1 v:v). The polar fraction was dried and derivatized using the method described above. The samples of jet (black amber) were extracted using the same protocol. Due to the lesser availability of leaves and jet and the lower percentage of extractable organic matter, we used the simplified fractionation described above in order to compare their biomarker composition with that of amber.

2.3.3. GC–MS

The analyses were performed on an Agilent 6850 GC coupled to an Agilent 5975C quadrupole mass spectrometer. Separation was achieved on a HP-5MS column coated with (5%-phenyl)-methylpolysiloxane (30 m × 0.25 mm, 0.25 µm film thickness). The operating conditions were as follows: 8 psi carrier pressure, initial temperature held at 40 °C for 1.5 min, increased from 40 °C to 150 °C at a rate of 15 °C/min, held for 2 min, increased from 150 °C to 255 °C at a rate of 5 °C/min, held constant for 20 min and finally increased to 300 °C at a rate of 5 °C/min. The sample was injected in the splitless mode with the injector temperature at 290 °C. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 40 to 700 Da. The temperature of the ion source was 230 °C and the quadrupole temperature was 150 °C. Data were acquired and processed using Chemstation software. Individual compounds were

identified by comparing their mass spectra with those of authentic standards and with published data (see Section 3.2).

3. Results and discussion

3.1. Infrared spectroscopy

The application of IR to the study of amber is well documented and constitutes a basic technique for the characterization of fossil resins (Langenheim, 1969; Grimalt et al., 1988; Alonso et al., 2000). Because of the inclination of all ambers and resins (even non-fossil resins) to show similar bulk infrared spectra (due to their common chemical functional groups), IR spectroscopy has strong limitations for the determination of their botanical origin (Yamamoto et al., 2006). The IR spectrum of the Cantabrian amber is consistent with those observed for other amber samples (Fig. S2; supplementary material) and could indicate that it is composed of a mixture of terpenoids and labdatriene copolymers. The band pattern is similar to the IR spectrum expected for the labdatrienes communic acid and biformene and their polymers, consistent with the stated macromolecular structure of amber (Villanueva-García et al., 2005) and with the terpenoid composition found (see Section 3.2). The weak band at 882 cm⁻¹ (Fig. S2, supplementary material) is characteristic of the exocyclic methylene moiety supporting the labdatriene input. The two types of amber samples found in the deposit show similar IR spectra.

3.2. Terpenoid composition of Cantabrian amber

The total extracts of the amber contain methylated naphthalenes (di-, tri- and tetramethylnaphthalenes) and di- and trimethyltetralins, sesquiterpenoids and bi- and tricyclic diterpenoids (Table 1). GC analysis of the bulk extract shows three different zones in the gas chromatogram (Fig. 3). The dominant compounds in the early

Table 1

Terpenoids and their diagenetic derivatives identified in bulk and chromatographic fractions of extracts from the ambers (types A and B) of the El Soplao deposit.

No ^a	Compound	Composition	MW	Relative abundance ^b	
				A	B
<i>Abietanes and Podocarpanes</i>					
1 (XVI)	16,17-Bisnorsimonellite	C ₁₇ H ₂₀	224	38.2	–
2 (XXII)	16,17,18-Trisnorabieta-8,11,13-triene	C ₁₇ H ₂₄	228	100	100
3	16,17,19-Trisnorabieta-8,11,13-triene	C ₁₇ H ₂₄	228	23.4	20.9
4 (XVII)	Retene	C ₁₈ H ₁₈	234	1.0	–
5	16,17-Bisnordehydroabietane	C ₁₈ H ₂₆	242	4.6	42.5
6	Simonellite	C ₁₉ H ₂₄	252	9.3	1.5
7 (XXVII)	14-Methyl-16,17-bisnordehydroabietane	C ₁₉ H ₂₈	256	2.5	6.9
8	1-Methyl-10,18-bisnorabieta-8,11,13-triene	C ₁₉ H ₂₈	256	–	6.6
9 (I)	18-Norabietatriene (Dehydroabietin)	C ₁₉ H ₂₈	256	45.8	14.0
10 (II)	19-Norabietatriene	C ₁₉ H ₂₈	256	3.6	4.1
11 (IV)	18-Norabieta-7,13-diene	C ₁₉ H ₃₀	258	9.7	–
12 (V)	Norabiet-13-ene	C ₁₉ H ₃₂	260	57.0	23.0
13 (III)	Fichtelite	C ₁₉ H ₃₄	262	5.8	d
14 (X)	12-Hydroxysimonellite	C ₁₉ H ₂₄ O	268	11.8	–
15 (II)	Dehydroabietane	C ₂₀ H ₃₀	270	40.3	–
16 (XXV)	16,17-Bisnordehydroabietic acid ^c	C ₁₈ H ₂₄ O ₂	272	d	d
17 (VIII)	Ferruginol	C ₂₀ H ₃₀ O	286	24.3	–
18 (XIII)	Callitricic acid ^c	C ₂₀ H ₂₈ O ₂	300	d	–
19 (XII)	Hinokiol ^c	C ₂₀ H ₃₀ O ₂	302	d	–
<i>Pimaranes and Isopimaranes</i>					
20 (XXI)	Pimaric acid ^c	C ₂₀ H ₃₀ O ₂	302	d	d
21 (XXII)	Isopimaric acid ^c	C ₂₀ H ₃₀ O ₂	302	d	d
22 (XXVI)	Pimar-8-en-18-oic acid ^c	C ₂₀ H ₃₂ O ₂	304	d	d
23	Pimaradiene	C ₂₀ H ₃₂	272	–	4.5
<i>Labdanes</i>					
24	14,15-Bisnorlabda-8,12-dien-18-oic acid ^c	C ₁₈ H ₂₈ O ₂	276	d	d
25 (XXXIV)	E-19-Noragathic acid	C ₁₉ H ₃₀ O ₂	290	10.6	3.1
26 (XXXIII)	Z-19-Noragathic acid	C ₁₉ H ₃₀ O ₂	290	3.7	1.5
27 (XXIX)	13-Dihydro-19-noragathic acid ^c	C ₁₉ H ₃₂ O ₂	290	d	–
28 (XXVIII)	13-Dihydroagathic acid	C ₂₀ H ₃₄ O ₃	322	11.8	–
<i>Other compounds</i>					
29	Ionene	C ₁₃ H ₁₈	174	37.2	48.4
30	Methylionene	C ₁₄ H ₂₀	188	13.0	34.2
31	Tetrahydroeudalene	C ₁₄ H ₂₀	188	16.0	8.3
32 (XXXVII)	Guaiazulene	C ₁₅ H ₁₈	198	7.6	1.5
33 (XXXVIII)	Cadalene	C ₁₅ H ₁₈	198	8.2	7.5
34	Drimane	C ₁₅ H ₂₈	208	3.6	14.8
35	Homodrimane	C ₁₆ H ₃₀	222	3.3	3.9
36 (XXXI)	2,5,8-Trimethyl-1-butyltetralin	C ₁₇ H ₂₆	230	79.1	57.3
37 (XXXII)	2,5,8-Trimethyl-1-isopentyltetralin	C ₁₈ H ₂₈	244	d	10.8
38 (XX)	Diaromatic totarane	C ₁₉ H ₂₄	252	1.0	–
39 (XI)	Totarol ^c	C ₂₀ H ₃₀ O	286	d	–

^a Roman numerals in parentheses refer to structures shown in Appendix A.^b Abundance relative to the major peak (100%) in the bulk extracts (GC–MS TIC). Occurrence is tabulated on compounds detected only after fractionation and derivatization (d: detected; -: not detected).^c Also analyzed as the TMS derivative.

elution range are α -ionene, methylionene, trimethylnaphthalene isomers, tetrahydroeudalene, calamenene isomers, drimane and homodrimane (identified after Dzou et al., 1999 and Sonibare and Ekweozor, 2004). The α -ionene, methylionene and drimanes may be derived from labdanes in the resin through degradation processes (Yamamoto et al., 2006; Pereira et al., 2009). Overall, these components are highly degraded diagenetic products that have no chemotaxonomic value due to their unrecognizable parent structures. The second section of the gas chromatogram is dominated by non-oxygenated bi- and tricyclic diterpenoids and the third section contains polar bi- and tricyclic diterpenoids. We did not find aliphatic lipids, hopanoids, fungal terpenoids or plant triterpenoids in the amber samples, discarding an angiosperm contribution and major contamination.

3.2.1. Abietane diterpenoids

The diterpenoids identified in the extracts belong to the abietane, pimarane/isopimarane and labdane structural classes (Fig. 3). These diterpenoids are typical of conifers (Otto and Wilde, 2001; Yamamoto et al., 2006), confirming such an origin for the Cantabrian ambers.

The abietane class terpenoids were identified by comparison of their mass spectra with those of standards or published in the literature (Czechowski et al., 1996; Otto and Simoneit, 2002; Otto et al., 2002; Hauteville et al., 2006; Cox et al., 2007), and comprised 18- and 19-norabieta-8,11,13-triene (I; chemical structures cited are shown in Appendix A), dehydroabietane (II), fichtelite (III), 18-norabieta-7,13-diene (IV) and norabiet-13-ene (V). The latter compound was tentatively identified by match with a mass spectrum in the literature (Hauteville et al., 2006), characterized by a molecular ion at m/z 260 and loss of an isopropyl group (m/z 217). 18-Norabieta-7,13-diene (IV) was identified only in sample A by a match with the published mass spectrum (Otto and Simoneit, 2002). This compound has been described as a decarboxylation product of abietic acid during diagenesis (Otto and Simoneit, 2002). In this case, the precursor molecule has not been found. The lack of a clear biological precursor for norabieta-7,13-diene (IV) suggests an alternative origin, possibly by double bond isomerization of unsaturated abietanes. This composition is consistent with the dominance of dehydroabietane and abietane geoterpenoids in the type A amber. The norabietatrienes (dehydroabietins) found in both amber

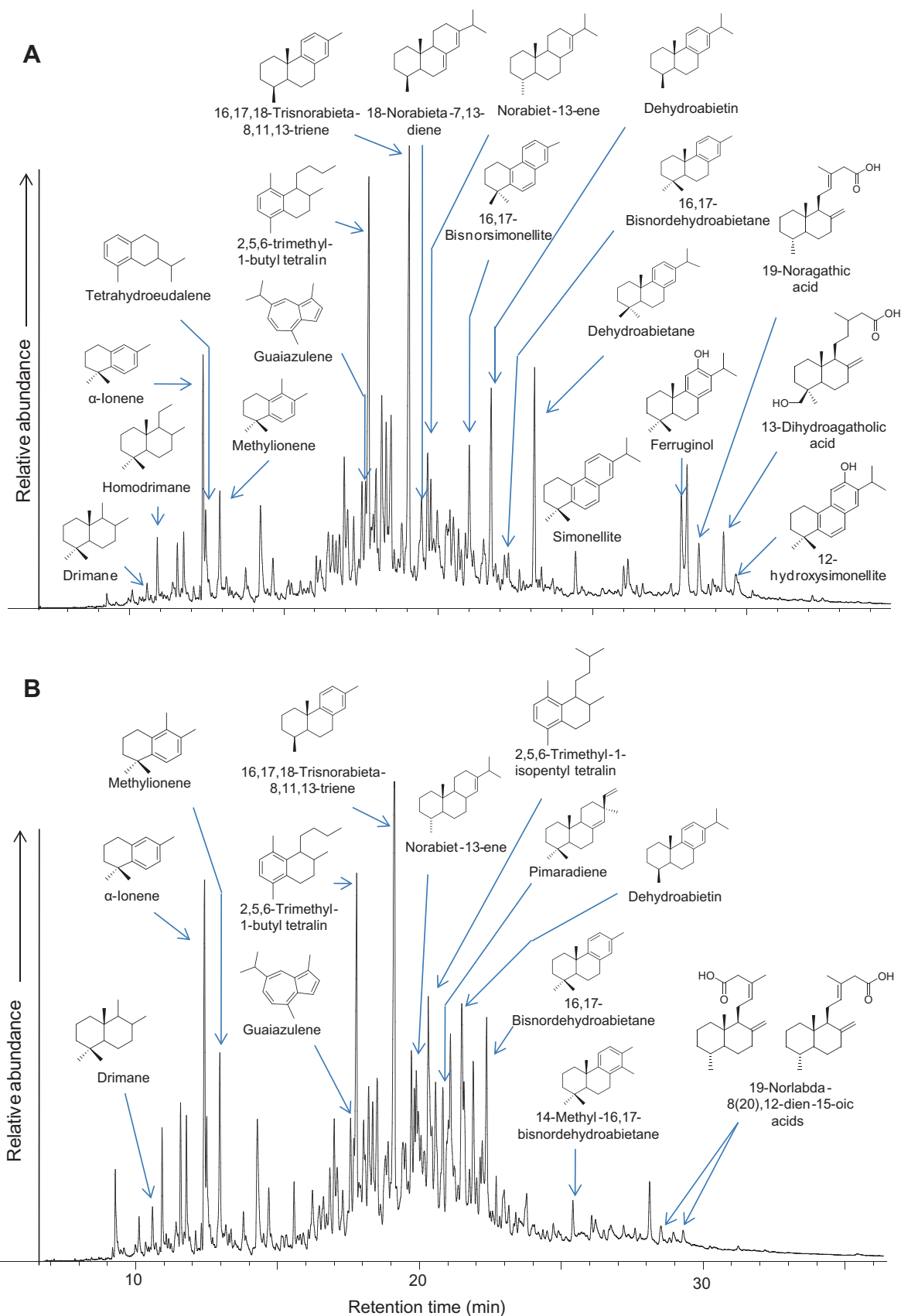


Fig. 3. GC-MS total ion current (TIC) traces of the underivatized total extracts of: (A) El Soplao blue amber, type A, (B) El Soplao yellow-blue amber, type B, with the main terpenoid compounds identified. Peaks not annotated are unidentified, tentatively identified or known compounds with little chemotaxonomic value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

samples could be derived from all abietane precursors by diagenetic alteration (Simoneit, 1986; Hautevelle et al., 2006).

Dehydroabietane (**II**) is a natural product of many Pinaceae resins (Otto et al., 2007) as well as some Cupressaceae resins. In these samples, dehydroabietane is a significant component in amber type A, whereas it is not detectable in amber type B, suggesting a different paleobotanical origin for both types of amber samples. The absence of abietic (**VI**) or dehydroabietic acids (**VII**) eliminates a Pinaceae contribution to the amber, because abietic acid is a major component of such resins and dehydroabietic acid, its major diagenetic derivative, is present in ambers derived from Pinaceae (Yamamoto et al., 2006).

Phenolic diterpenoids occur in polar fraction B of the type A amber (Fig. 4), with a dominance of ferruginol (**VIII**) and its oxidation products 6,7-dehydroferruginol (**IX**) and 12-hydroxysimonellite (**X**) and totarol (**XI**) (Otto and Simoneit, 2001; Otto et al., 2002). Ferruginol and 12-hydroxysimonellite are also identifiable (underivatized) in the bulk extract as part of the main components of the amber (Fig. 3). The presence of these phenolic diterpenoids is of significant chemosystematic value, as ferruginol is an abundant natural product in extant conifers of the families Cupressaceae and Podocarpaceae and can be used as a characteristic biomarker of these families (Otto and Simoneit, 2001; Marynowski et al., 2007). A minor amount of hinokiol (3-hydroxyferruginol, **XII**; Fig. 5A) was also found in the type A amber. Hinokiol has been described from Cupressaceae (Otto et al., 2002; Cox et al., 2007). There is no reported presence of phenolic diterpenoids in modern Araucariaceae (Cox et al., 2007), but Otto and Wilde (2001) cited the occurrence of ferruginol in *Araucaria*. To avoid this ambiguity and to test this finding under our experimental conditions, resins of *Agathis* sp. and *Araucaria* sp. were analyzed and ferruginol was not detected in any Araucariaceae resins. Hence, these results, coupled with the absence of kaurane or phyllocladane diterpenoids, the

presence of totarol (see below) and the fossil record of the deposit, suggest that Araucariaceae did not contribute to the main type of amber found at the studied deposit (type A). On the other hand, we did not find phenolic abietanes in the type B amber sample. This fact, taken together with the presence of dehydroabietane in sample A and its absence in sample B, constitutes the main chemotaxonomic difference between the two types of samples. A significant relationship between amber type A and modern Cupressaceae is the presence of a low quantity of callitricic acid (**XIII**) which is an epimer of dehydroabietic acid (**VII**, Fig. 5). The difference in retention time with dehydroabietic acid and the higher relative intensity of the ion at m/z 357 (M-CH₃) versus the molecular ion in callitricic acid are distinctive features between the two epimers used here for the identification of the acid (Van den Berg et al., 2000; Cox et al., 2007). Callitricic acid has a higher chemotaxonomical value than dehydroabietic acid due to its scarcity. In modern conifer resins, the synthesis of callitricic acid seems to be restricted to certain genera of the Cupressaceae family and it was also found in Cenomanian amber from the Raritan Formation (New Jersey, USA), suggesting a relationship with Cupressaceae (Anderson, 2006).

Degradation of phenolic diterpenoids could lead to the abietane geoterpenoids found in the type A amber (Otto et al., 1997; Otto and Simoneit, 2001; Stefanova et al., 2002). Hautevelle et al. (2006) and Yamamoto et al. (2006) discussed the diagenetic pathways of abietane class bioterpenoids, suggesting that 18-norferruginol (**XIV**) could be the precursor of dehydroabietin, and ferruginol (**VIII**) could lead to 12-hydroxysimonellite (**X**), simonellite (**XV**), 16,17-bisnorsimonellite (**XVI**) and retene (**XVII**), all found in the type A amber. Under the anaerobic depositional conditions of the amber (Najarro et al., 2009), we could not disregard redox reactions that lead to the actual composition found (Pereira et al., 2009). If, as in some modern Cupressaceae genera (i.e. *Cupressus*; Fig. S3, supplementary material), the original proportion of ferruginol (**VIII**) was high, its

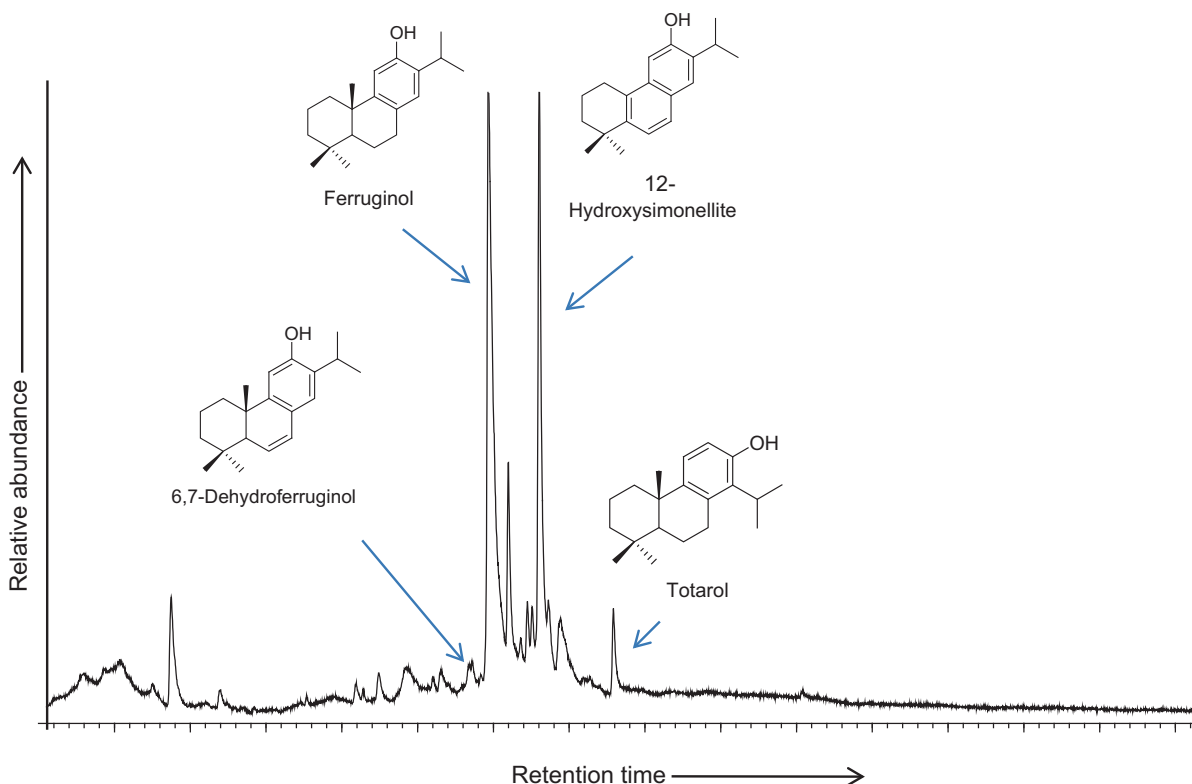


Fig. 4. GC-MS TIC trace of fraction B resulting from the column separation of the polar fraction of amber A. This fraction contains the partially purified phenolic terpenoids. Compounds are analyzed as the TMS derivatives.

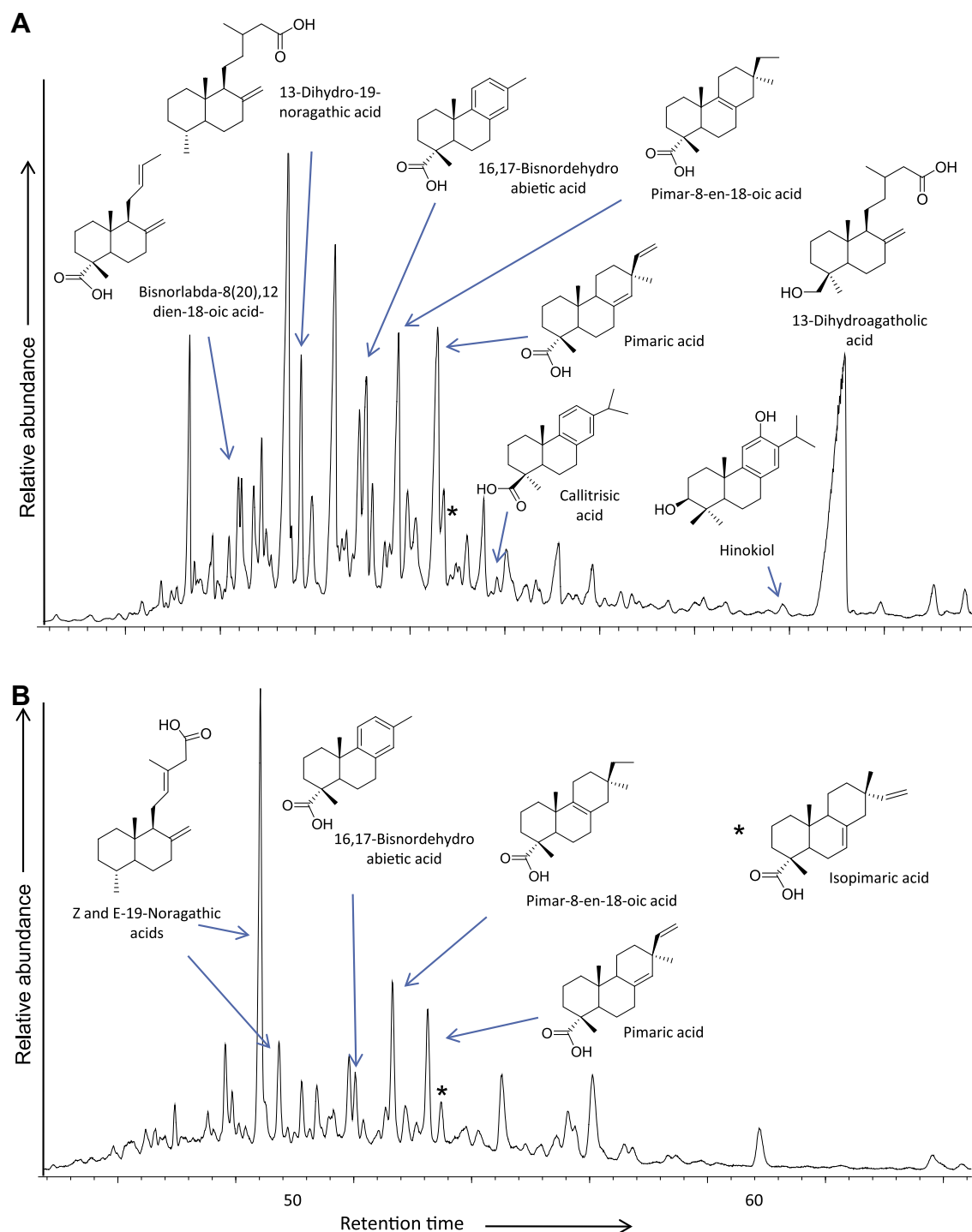


Fig. 5. GC–MS TIC traces of the polar fractions C of: (A) amber A and (B) amber B. This fraction contains mainly pimaric and labdenic acids. The figure shows the identified compounds in the fraction. Unlabelled peaks are considered as unidentified due to the lack of standards and published references or databases with details of their mass spectra. Compounds are analyzed as the TMS derivatives.

diagenesis could ultimately have generated dehydroabietane (**II**) and simonellite (**XV**), both significant in the type A amber.

3.2.2. Totarol

Totarol (**XI**), a tricyclic diterpenoid phenol, is considered as a confirmatory chemotaxonomic marker for Cupressaceae and Podocarpaceae, even at low concentrations (Le Métayer et al., 2008; Stefanova and Simoneit, 2008). Totarol is detectable in the bulk extract using the characteristic mass fragments of m/z 271 and 286. The identification is unambiguous in amber sample A after purification of the phe-

nolic fraction of the bulk extract and analysis as trimethylsilyl derivatives (Fig. 4). Due to the similarities between the mass spectra of phenolic diterpenoids, the retention time and mass spectrum of totarol (**XI**) were determined using a standard (Sigma–Aldrich). The presence of totarol suggests a relationship between the palaeobotanic origin of the amber and extant Cupressaceae or Podocarpaceae. To test this possibility, the phenolic diterpenoids of the amber were compared with those from a modern Cupressaceae (*Cupressus arizonica*; Fig. S3, supplementary material). Both extracts contain ferruginol (**VIII**), totarol (**XI**) and hinokiol (**XII**) as the main phenolic

diterpenoids, but semperviol (**XVIII**) has not been observed. A difference between the assemblage of polar terpenoids from *C. arizonica* and the amber is the presence of sugiol (**XIX**) and the lack of callitricic acid (**XIII**) in the former. We identified a diaromatic totarane (**XX**) as a possible diagenetic product of totarol (**XI**), which may be derived by a parallel diagenetic pathway as simonellite (**XV**) from ferruginol (**VIII**) (Otto et al., 1997). In accord with the phenolic abie-

tane composition, totarol is not detectable in the type *B* amber, confirming that both amber types found in the Cantabria deposits differ in their biological origins.

3.2.3. Pimarane/isopimarane diterpenoids

Polar fraction C of amber contains low amounts of pimaric (**XXI**) and isopimaric (**XXII**) acids (Fig. 6). Diagenesis of pimarane

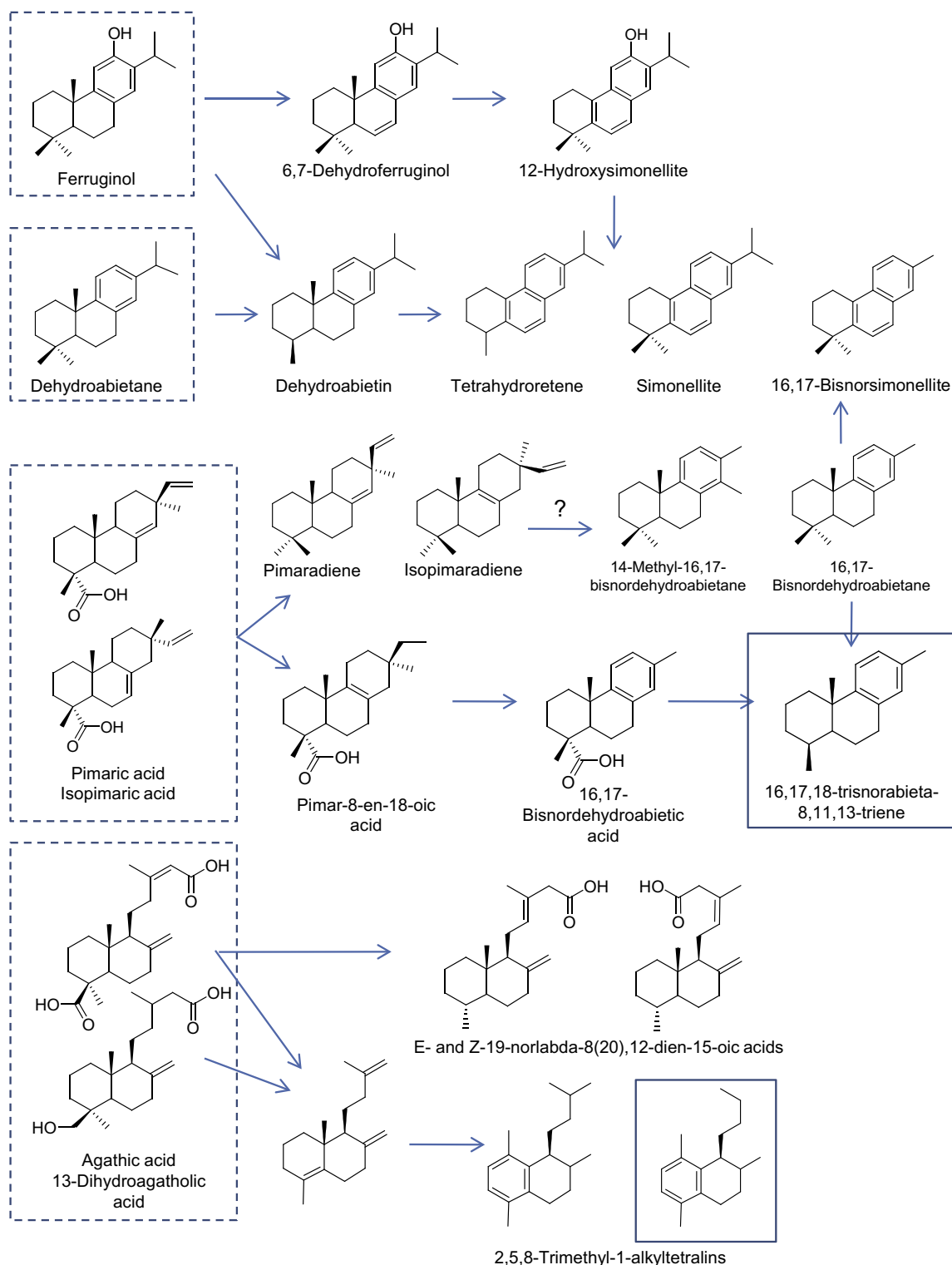


Fig. 6. Proposed diagenetic pathways for the diterpenoid precursors from the Cantabrian ambers (based on Otto and Simoneit (2002), Stefanova et al. (2002), Hauteville et al. (2006), and Pereira et al. (2009)). Dotted box: biological precursors; solid box: major terpenoid found in the samples.

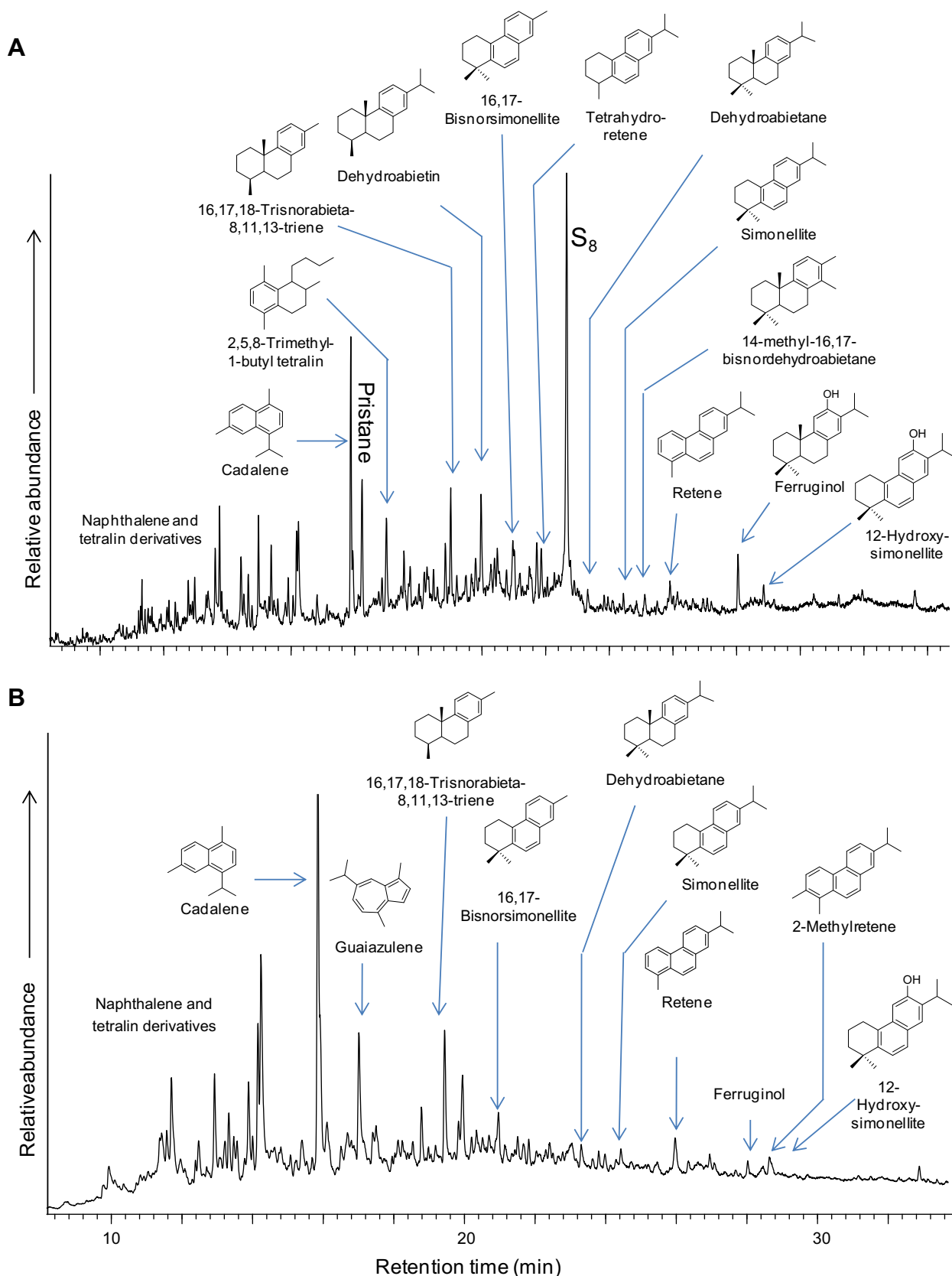


Fig. 7. GC-MS TIC traces of the total extracts from: (A) *Frenelopsis* leaves and (B) jet (black amber), showing the identified biomarkers.

diterpenoids could be one of the possible origins of 16,17,19-trisnorabieta-8,11,13-triene (XXIII), a major compound identified in the amber samples. Another possible origin for this compound is by diagenesis of dehydroabietane and other abietane related terpe-

noids (Fig. 6; Otto et al., 2002; Pereira et al., 2009). Due to the widespread distribution of the pimaric/isopimaric acids, the chemotaxonomical interpretation of their presence in the Cantabrian ambers must be taken with caution and comparisons with

other markers and samples should be made. Continuing the comparison with extant conifers, pimaric and isopimaric acids constitute the main tricyclic resin acids present in the conifer families Cupressaceae, Araucariaceae and Podocarpaceae (Otto and Wilde, 2001). Taking into account a possible molecular relationship between the botanical source of Cantabrian amber *A* and modern Cupressaceae, if pimaric acid (XXI) and isopimaric acid (XXII) were the main resin acids in the precursor resin of these ambers, diagenetic degradation to 16,17,18-trisnorabieta-8,11,13-triene (XXIII) is consistent with the dominance of the pimarane resin acid compounds in the extract, as loss of the vinyl moiety at C-13, with concomitant aromatization and decarboxylation at C-4 generates this predominant isomer. Following this pathway, the presence of a related molecule to 16,17,18-trisnorabieta-8,11,13-triene with a C-13 ethyl group (i.e. 16,18-bisnorabieta-8,11,13-triene, XXIV) should be expected as well, but we failed to detect such a compound. Otto et al. (2002) reported a significant presence of that geoterpenoid (XXIV) in fossil resin of the Lower Cretaceous *Tritaeonia linkii* (Miroviaceae), but due to the absence of precursor bioterpenoids, the assignment to a specific taxon was unclear. Recent work of Pereira et al. (2009) on Cretaceous amber from Brazil, inferred some intermediates of this diagenetic route (Fig. 6), namely 16,17-bisnordehydroabietic acid (XXV), pimar-8-en-18-oic acid (XXVI) and 14-methyl-16,17-bisnordehydroabietane (XXVII), that are also present in the type *A* amber (Figs. 3–5). Work is in progress in our laboratory in order to confirm this hypothetical pathway, as the formation of 14-methyl-16,17-bisnordehydroabietane by rearrangement of a pimaradiene or abietane precursor has not been demonstrated to date.

3.2.4. Labdane diterpenoids

Labdanoic acids and other labdane derivatives are common components in all conifers and are therefore non-specific biomarkers (Otto and Wilde, 2001). 13-Dihydroagatholic acid (XXVIII) is the predominant labdanoic acid present in the polar fraction of amber *A* (Fig. 5). This acid could be a precursor molecule preserved that constitutes the chemotaxonomic difference between the two paleobotanical resin producers, as it is not detectable in the extract of amber *B*. 13-Dihydro-19-noragathic acid (XXIX), found in sample *A*, could be formed from the 13-dihydroagatholic acid (XXVIII) precursor by loss of the C-19 hydroxymethyl group at C-4 or from the agathic acid (XXX) precursor by C-19 decarboxylation at C-4, respectively. The diagenetic transformation of the major labdanoic acids may be the source of the 2,5,8-trimethyl-1-alkyltetralins, ionenes and drimanes found in the samples (Fig. 3). The MS fragmentation pattern of the major compound of this family, i.e. 2,5,8-trimethyl-1-butyltetralin (XXXI) with a molecular ion at m/z 230, shows a butyl loss (57 da) from the saturated ring to form an m/z 173 fragment (see Fig. S4, supplementary material). Another homologue of this compound group is 2,5,8-trimethyl-1-isopentyltetralin (XXXII), which is significant in amber type *B* but only occurs in trace amounts in amber *A*. The degradation pathway leading to XXXII could be decarboxylation at C-4 of a labdanoic acid precursor (i.e. agathic acid, XXX), followed by aromatization of ring *A* with methyl migration from C-10 to C-1 and decarboxylation of C-15 (Fig. 6). These compounds were also reported from Brazilian ambers (Pereira et al., 2009). The difference found between the ambers could be indicative of differential labdanoic acid compositions in the original resins. Another source of these molecules may be the degradation of the labdane macromolecular structure of amber due to particular conditions that prevailed in the Cantabrian deposits (see below). Other diagenetic degradation products of labdanoic acids found in both amber samples are *Z*- and *E*-19-norlabda-8(20),12-dien-15-oic acids (XXXIII and XXXIV, respectively) and bisnorlabda-8(20),12-dien-18-oic acid (XXXV) (Otto and Simoneit, 2002). It is not possible to identify

all peaks found in the polar fractions of the amber extracts due to the lack of references and possible precursors.

During burial, the El Soplao and Reocín amber deposits suffered the influence of hydrothermal fluids related to the La Florida-Reocín Pb–Zn mine mineralization. As a consequence, we cannot discard the possibility of alternative transformation routes leading to the unusual compounds found in the amber. These alternative transformations must be added to the lack of information about the possible chemotaxonomy of extinct Cheirolepidiaceae. Despite these uncertainties, which result in the presence of some unidentified compounds, the low maturation (cf. vitrinite reflectance data, supplementary material) and excellent preservation of the organic compounds in the amber allows us to use the components of the extract to obtain chemotaxonomic information. We suggest that the tricyclic diterpenes originally biosynthesized by the main botanical precursor of amber *A* were dominated by phenolic abietanes, pimarane resin acids and totarol. The bicyclic diterpenes probably contained a high proportion of labdanoic acids, in particular 13-dihydroagatholic acid (XXVIII) and communic acid (XXXVI), whose polymerization leads to the typical macromolecular structure of ambers.

3.3. Terpenoid composition of co-occurring fossil leaves and paleochemotaxonomic aspects

Due to the lack of Cupressaceae representatives in all the outcrops of Las Peñas Formation (Fig. 1) and the excellent preservation and dominance of *Frenelopsis* material, the comparison of the terpenoid assemblage of these plant remains with those found in the amber may help to confirm the botanical origin of the fossil resin and to understand the chemosystematics of the extinct family Cheirolepidiaceae. Since no amber associated with this family has been documented to date (Bray and Anderson, 2008; Pereira et al., 2009), the inclusion of *Frenelopsis* genera as a possible source of one of the amber types found in the El Soplao deposit has to be considered. Previous reports relating a possible botanical origin of ambers to Cheirolepidiaceae should be mentioned (Gomez et al., 2002b; Roghi et al., 2006). Macrofossil evidence of two potential conifer resin producers was found by Najarro et al. (2009) in the study zone: *Frenelopsis* (Cheirolepidiaceae family) and *Mirovia* (Miroviaceae). Also, the palynological record shows a contribution from the Araucariaceae family, but no meso- or macrofossils of this family have been recognized yet. As Anderson (2006) pointed out, the correlation between plant fossil evidence and co-deposited amber should be taken with caution since the major resin producer could be a minor species in the ecosystem. As we find two different potential palaeobotanical contributors for the ambers of the El Soplao deposit, all the types of plant macrofossil remains identified in the deposit were examined separately in order to establish possible chemosystematic relationships.

Overall, despite the increase of aromatized derivatives such as retene, the diterpene speciation in the *Frenelopsis* leaves shows that all the main components are shared with the type *A* ambers from El Soplao (Fig. 7A). Cadalene (XXXVII) and 16,17,18-trisnorabieta-8,11,13-triene (XXIII) are among the biomarkers detected in the fossil leaves of *Frenelopsis*. The diagenetic processes undergone by terpenoids from the fossil leaves are consistent with those observed in sediments, as the leaves are not protected by the polymeric structure of the amber. The formation of aromatic derivatives may be governed by clay catalysis or other abiotic processes in soils and sediments (Otto et al., 2007). Also, the aromatic abietanes may be generated under aerobic conditions, consistent with the major presence of pristane and the lack of phytane (Peters et al., 2005). The presence of norabietanes is consistent with the diagenetic processes for terpenoids described by Frenkel and Heller-Kallai (1977). 14-Methyl-16,17-bisnordehydroabietane (XXVII)

and 2,4,8-trimethylalkyltetralins (e.g. **XXXI**), described above, are also present in the *Frenelopsis* leaves. The phenolic abietane ferruginol (**VIII**) and its derivative 12-hydroxysimonellite (**X**) are key biomarkers also found in the *Frenelopsis* leaves. This result is consistent with the presence of ferruginol in Cenomanian *Frenelopsis alata* (Nguyen Tu et al., 2000a). This evidence, together with the absence of ferruginol in *Mirovia* leaves (Fig. 8), suggests that *Frenelopsis* could be one of the botanical origins for the Cantabrian amber. The terpenoid composition found in *Mirovia* leaves is dominated by oxidized non-specific abietane terpenoids (mainly simonellite and retene).

The *Frenelopsis* leaves and the amber of the El Soplao deposit are largely associated with jet (black amber). The analysis of jet extract shows a composition dominated by cadalene and alkyl derivatives of naphthalene and tetralin. The identifiable terpenoids include aromatized abietanes and ferruginol. Fractionation and derivatization and GC–MS of jet extract showed the presence of ferruginol and totarol, suggesting that jet has the same botanical origin as the main type A amber in the deposit.

The azulene hydrocarbon derivative guaiazulene (**XXXVIII**), an isomer of cadalene (**XXXVII**), with a strong blue color and purple-blue fluorescence, is found in low amounts in all type A ambers, *Frenelopsis* leaves and jet, suggesting a common origin from sesquiterpenoids synthesized by Cheirolepidiaceae. Guaiazulene is a common compound with low chemosystematic value, but the presence of this hydrocarbon in amber has not been reported to date. The significant quantity of this compound in the El Soplao samples could be at the cause for the characteristic blue–purple tinge of these ambers. Although the relationships between Cheirolepidiaceae and extant conifers are unclear (Bray and Anderson, 2008; Pereira et al., 2009), a morphological and histological correlation between Cheirolepidiaceae and Cupressaceae has been established (Daviero et al., 2001; Farjon, 2008). Moreover, Nguyen Tu et al. (2000b) have observed a resemblance between the lipid composition of *Frenelopsis alata* and *Tetraclinis articulata*, a representative of Cupressaceae. The presence of ferruginol in *Frenelopsis* (Nguyen Tu et al., 2000b and the present data) confirms the hypothesis of a possible relationship between *Frenelopsis* and the

Cupressaceae family. Moreover, the presence of 13-dihydroagathic acid (**XXVIII**) in the amber and the overall biomarker assemblage show a similarity to extant *Cupressus* genera (see Fig. S4, supplementary material). The resemblance in the chemical composition between *Frenelopsis* and Cupressaceae representatives may be due to convergence, as it has been demonstrated that the physical similarities between these taxa resulted from convergence rather than phylogenetic connection (Broutin and Pons, 1975; Alvin, 1982). The evolutionary changes in the biochemistry of terpenoids since the synthesis of the parent resin of amber to the modern conifers are unknown. Consequently, we should consider that the lack or presence of certain compounds in a correlation with extant conifers is informative, and that detailed biomarker compositions of extinct conifer fossils, complemented by morphological and histological relationships, are necessary to establish a definite evolutionary relationship. Keeping this in mind, the paleobotanical considerations suggested by our data obtained on macrofossil plant samples and amber types can be summarized as follows:

- The absence of abietic and dehydroabietic acid in both types of amber samples excludes an origin from resin of the Pinaceae family. Also, the absence of plant triterpenoids and labdanoic acids eliminates a contribution from angiosperms (Anderson et al., 1992; Yamamoto et al., 2006).
- The presence of phenolic terpenoids (ferruginol, totarol and hinokiol) in the type A amber indicates the conifer families Cupressaceae and Podocarpaceae as possible biological precursors, and rejects the Araucariaceae family. The presence of callitricic acid (**XIII**) reinforces the biochemical relationship between the parent resin of amber and modern Cupressaceae. The plant macrofossil record in the deposit shows that there are no representatives of Cupressaceae or Podocarpaceae among the possible resin producers (Najarro et al., 2009). The co-occurrence of key terpenoids (e.g. ferruginol), between amber A and fossil tissue of *Frenelopsis* suggests that this amber could be derived from *Frenelopsis* (Cheirolepidiaceae).

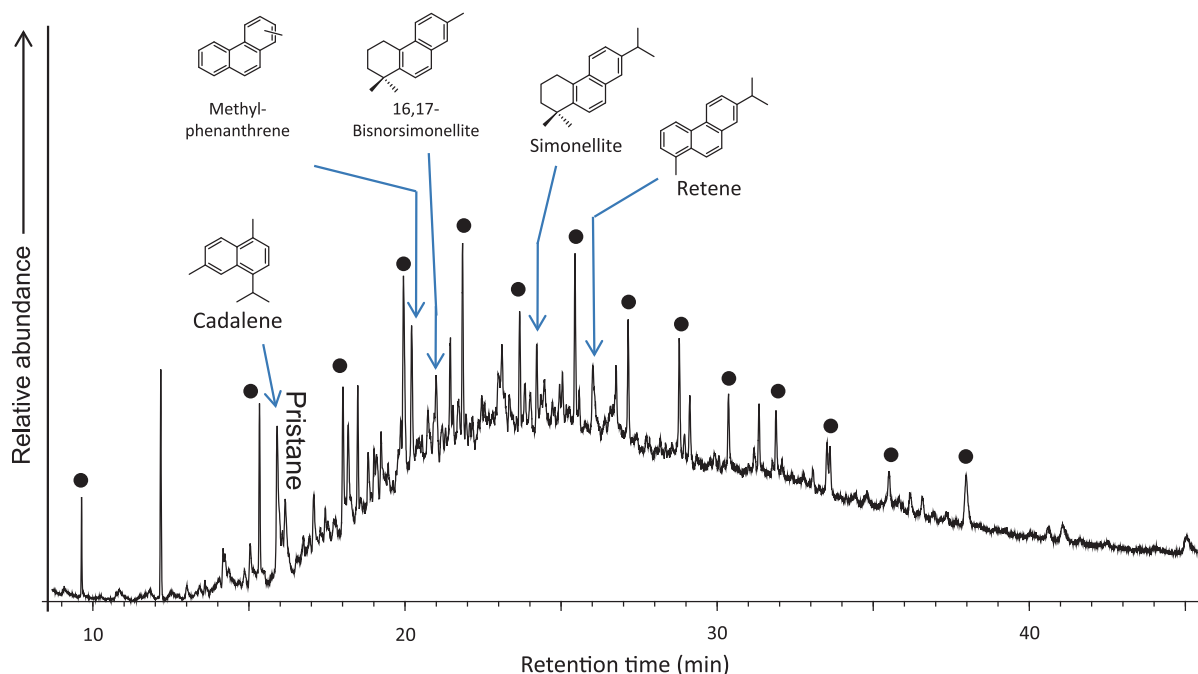


Fig. 8. GC–MS TIC trace of the total extract of *Mirovia* sp. leaves found in the amber deposit at El Soplao showing the identified biomarkers. Solid dots: *n*-alkanes (last dot: C₃₀).

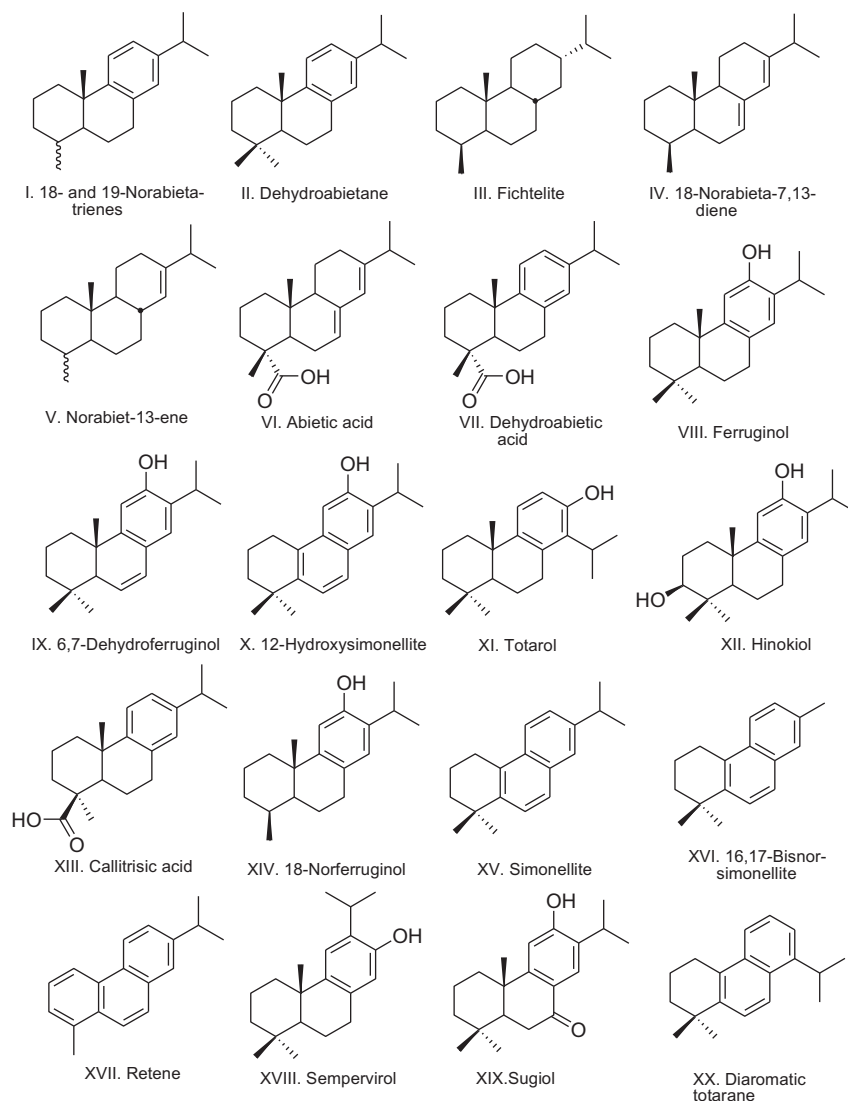


Fig. A1. Chemical structures cited in the text.

c. The overall terpenoid composition of the type *B* amber is comprised of non-specific conifer biomarkers. The absence of phenolic terpenoids and of 13-dihydroagatholic acid (XXVIII), together with the presence of major amounts of diagenetic products of pimarane-type diterpenoids, saturated and unsaturated norabietanes, and alkyltetralins point to a different biological origin. A paleobotanical source for this type of amber could not be determined on the basis of its biomarker composition.

4. Conclusions

Analysis of the polar diterpenoids of Cretaceous ambers from El Soplao (Cantabria, Spain) indicates that two resin producers contributed to the amber record. The main parent resin (type *A*) originally contained phenolic abietanes (dominated by ferruginol), totarol, dehydroabietane and pimaric/isopimaric acids. The dominant resin acids found are 13-dihydroagatholic and bisnordehydroabietic acids, with various other alteration products and a minor quantity of callitrisic acid. This composition suggests a biochemical relation with the resin of extant Cupressaceae. The second parent resin (type *B*) contains pimaric/isopimaric acids as the only identifiable biological precursors preserved. The phenolic diterpenoids present in the

samples (type *A*), the lack of phyllocladane/kaurane-type terpenoids and the absence of macrofossil plant remains exclude a significant contribution of Araucariaceae to the amber. Diagenetic products of the pimarane/abietane and labdane class terpenoids constitute the main geoterpenoids extractable from the amber of El Soplao. Insights from petrographic characterization of coal macerals provide a correlation between temperature, time and level of organic diagenesis, indicating only a moderate degree of diagenetic alteration during burial. This is consistent with the high level of preservation of the natural product diterpenoids and their direct diagenetic derivatives. The sedimentological relationships and chemotaxonomical observations suggest that one source of the amber may be the extinct *Frenelopsis* (Cheirolepidiaceae).

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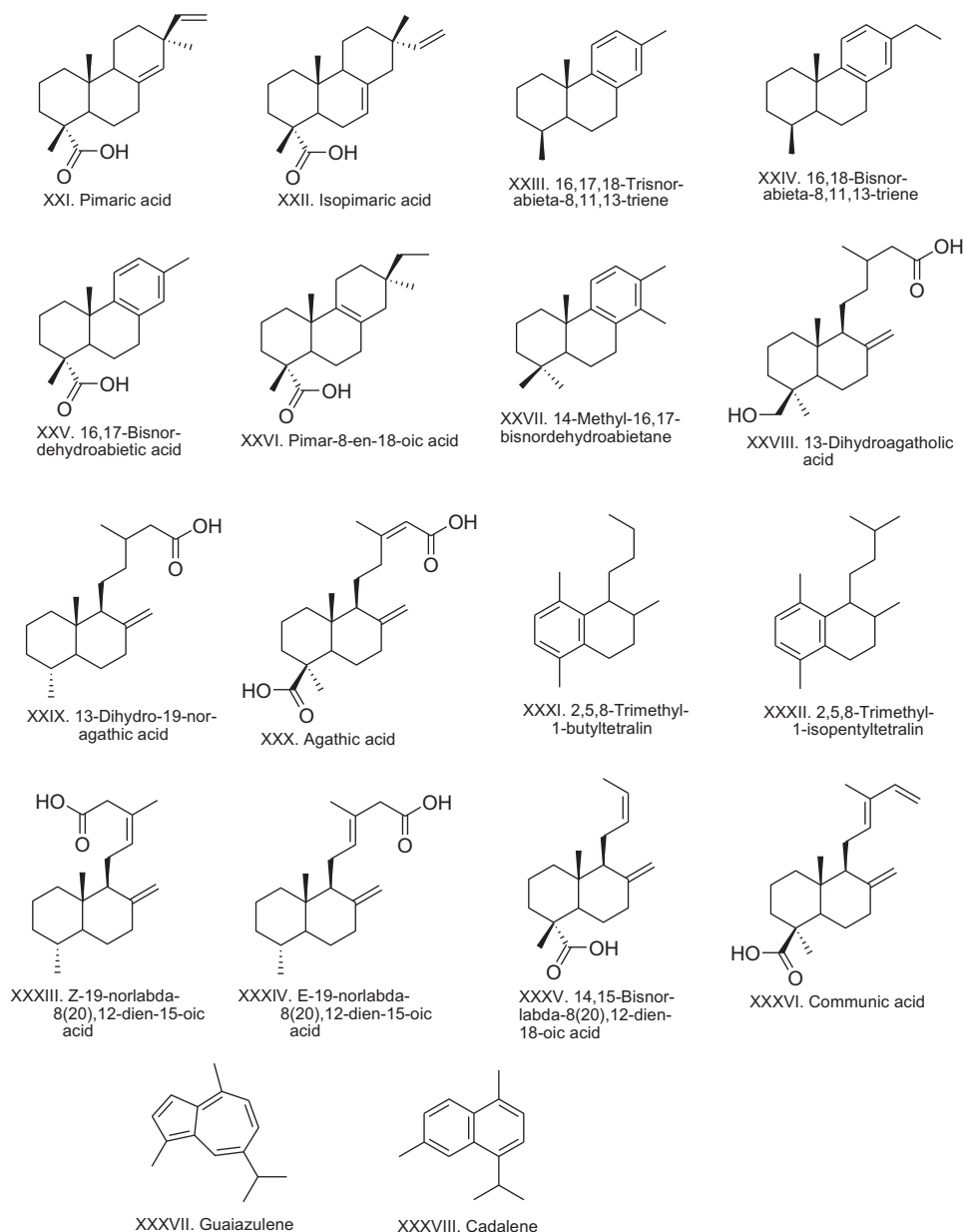


Fig. A1 (continued)

(Regional Minister of the Cantabria Government) and F. Unzué (manager of El Soplao Cave and Territory) for their support and promotion of the study of the new amber deposit at El Soplao. Thanks should go to Antonino Bueno Yanes for providing Reocín amber samples. We thank the Centro de Astrobiología (CSIC-INTA) and Instituto de Técnica Aeroespacial “Esteban Terradas”, where all chemical analyses were performed. This study is framed in a collaborative agreement among IGME, SIEC S.A. and the Cantabria Government (Consejería de Cultura, Turismo y Deportes), and is a contribution to IGME project 491-CANOA 53.6.00.12.00: “Investigación científica y técnica de la Cueva de El Soplao y su entorno geológico”, and DGI project CGL2008-01237/BTE (MICINN, Spanish Government).

Appendix A

See Fig. A1.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.orggeochem.2010.06.013](https://doi.org/10.1016/j.orggeochem.2010.06.013).

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References

- Alonso, J., Arillo, A., Barron, E., Carmelo-Corral, J., Grimalt, J., Lopez, J.F., Lopez, R., Martínez-Delclós, X., Ortuño, V., Peñalver, E., Trincao, P.R., 2000. A new fossil resin with biological inclusions in Lower Cretaceous deposits from Alava (Northern Spain, Basque-Cantabrian Basin). *Journal of Paleontology* 74, 158–178.
- Alvin, K.L., 1982. Cheirolepidiaceae: biology, structure and paleoecology. *Reviews in Palaeobotany and Palynology* 37, 71–98.
- Alvin, K.L., Hlušík, A., 1979. Modified axillary branching in species of the fossil genus *Frenelopsis*: a new phenomenon among conifers. *Botanical Journal of the Linnean Society* 79, 231–241.

- Anderson, K.B., 2006. The nature and fate of natural resins in the geosphere. XII. Investigation of C-ring aromatic diterpenoids in Raritan amber by pyrolysis-GC-matrix isolation FTIR-MS. *Geochemical Transactions* 7, 2–7.
- Anderson, K.B., Crelling, J.C., 1995. Amber, Resinite and Fossil Resins. ACS Symposium Series 617. American Chemical Society, Washington, DC, USA.
- Anderson, K.B., Winans, R.E., Botto, R.E., 1992. The nature and fate of natural resins in the geosphere – II. Identification, classification and nomenclature of resinites. *Organic Geochemistry* 18, 829–841.
- Barrón, E., Comas-Rengifo, M.J., Elorza, L., 2001. Contribuciones al estudio palinológico del Cretácico Inferior de la Cuenca Vasco-Cantábrica: los afloramientos ambarígenos de Peñacerrada (España). *Coloquios de Paleontología* 52, 135–156.
- Bray, P.S., Anderson, K.B., 2008. The nature and fate of natural resins in the geosphere XIII: a probable pinaceous resin from the early Cretaceous (Barremian), Isle of Wight. *Geochemical Transactions* 9, 3–9.
- Bray, P.S., Anderson, K.B., 2009. Identification of Carboniferous (320 million year old) Class Ic amber. *Science* 326, 132–134.
- Broutin, J., Pons, D., 1975. Nouvelles précisions sur la morphologie et la phytodermologie de quelques rameaux du genre *Frenelopsis* Schenk. In: *Comptes Rendus du 100ème Congrès National des Sociétés Savantes*, Paris, vol. II, 1975, pp. 29–46.
- Chaler, R., Grimalt, J.O., 2005. Fingerprinting of Cretaceous higher plant resins by infrared spectroscopy and gas chromatography coupled to mass spectrometry. *Phytochemical Analysis* 16, 446–450.
- Cox, R.E., Yamamoto, S., Otto, A., Simoneit, B.R.T., 2007. Oxygenated di- and tricyclic diterpenoids of southern hemisphere conifers. *Biochemical Systematics and Ecology* 35, 342–362.
- Czechowski, F., Simoneit, B.R.T., Sachanbinski, M., Chojcan, J., Wolowicz, S., 1996. Physicochemical structural characterization of ambers from deposits in Poland. *Applied Geochemistry* 11, 811–834.
- Daviero, V., Gomez, B., Philippe, M., 2001. Uncommon branching pattern within conifers: *Frenelopsis turolensis*, a Spanish Early Cretaceous Cheirolepidiaceae. *Canadian Journal of Botany* 79, 1400–1408.
- Delclós, X., Arillo, A., Peñalver, E., Barrón, E., Soriano, C., López del Valle, R., Bernárdez, E., Corral, C., Ortuño, V.M., 2007. Fossiliferous amber deposits from the Cretaceous (Albian) of Spain. *Comptes Rendus Palevol* 6, 135–149.
- Dzou, L.L., Holba, A.G., Ramon, J.C., Moldowan, J.M., Zinniker, D., 1999. Application of new diterpane biomarkers to source, biodegradation and mixing effects on Central Llanos Basin oils, Colombia. *Organic Geochemistry* 30, 515–534.
- Farjon, A., 2008. *The Natural History of Conifers*. Timber Press, New York.
- Frenkel, M., Heller-Kallai, L., 1977. Aromatization of limonene – a geochemical model. *Organic Geochemistry* 1, 3–5.
- García-Mondéjar, J., Aguirrezabala, L.M., Aranburu, A., Fernández-Mendiola, P.A., Gómez-Pérez, I., López-Horgue, M., Rosales, I., 1996. Aptian–Albian tectonic pattern of the Basque–Cantabrian Basin (northern Spain). *Geological Journal* 31, 13–45.
- Gomez, B., Martín-Closas, C., Barale, G., Solé de Porta, N., Thévenard, F., Guignard, G., 2002a. *Frenelopsis* (Coniferales: Cheirolepidiaceae) and related male organ genera from the Lower Cretaceous of Spain. *Palaeontology* 45, 997–1036.
- Gomez, B., Bamford, M., Martínez-Delclós, X., 2002b. Lower Cretaceous plant cuticles and amber (Kikwood Formation, South Africa). *Comptes Rendus Palevol* 1, 83–87.
- Gomez, B., Thévenard, F., Fantin, M., Giusberti, L., 2003. Late Cretaceous plants from the Bonarelli Level of the Venetian Alps, northeastern Italy. *Cretaceous Research* 23, 671–685.
- Gradstein, F.M., Ogg, J.G., Smith, A.G., 2004. *A Geologic Time Scale 2004*. Cambridge University Press.
- Grimalt, J.O., Simoneit, B.R.T., Hatcher, P.G., Nissenbaum, A., 1988. The molecular composition of ambers. *Organic Geochemistry* 13, 677–690.
- Hauteville, Y., Michels, R., Malartre, F., Trouiller, A., 2006. Vascular plant biomarkers as proxies for palaeoflora and palaeoclimatic changes at the Dogger/Malm transition of the Paris Basin (France). *Organic Geochemistry* 37, 610–625.
- Hines, F.M., 1985. Sedimentation and tectonics in north-west Santander. In: Milá, M.D., Rosell, J. (Eds.), 6th European Regional Meeting, Excursion Guidebook. International Association of Sedimentologists, pp. 371–398.
- Lambert, J.B., Johnson, S.C., Poinar Jr., G.O., 1996. Nuclear magnetic resonance characterization of Cretaceous amber. *Archaeometry* 38, 325–335.
- Langenheim, R.H., 1969. Amber: a botanical inquiry. *Science* 163, 1157–1169.
- Le Métayer, P., Schaeffer, P., Adam, P., Albrecht, P., Roussé, S., Düringer, P., 2008. An unprecedented condensation pathway leading to the formation of phenolic C₄₀ bis-diterpenoids in sediments from the Lower Oligocene of the Rhine Rift Valley. *Organic Geochemistry* 39, 658–675.
- Le Pichon, X., Sibuet, J.C., 1971. Western extension of boundary between European and Iberian plates during the Pyrenean orogeny. *Earth and Planetary Science Letters* 12, 83–88.
- López-Horgue, M.A., Aranburu, A., Fernández-Mendiola, P.A., García-Mondéjar, J., 2001. Facies estuarinas en el Albiense superior de Cabo Quintres (Cantabria, región Vasco-Cantábrica occidental). *Geogaceta* 30, 75–78.
- Marynowski, L., Otto, A., Zaton, M., Philippe, M., Simoneit, B.R.T., 2007. Biomolecules preserved in ca. 168 million year old fossil conifer wood. *Naturwissenschaften* 94, 228–236.
- Menor-Salván, C., Najarro, M., Rosales, I., Velasco, F., Tornos, F., 2009. Quimiotaxonomía y Origen Botánico del Ámbar de El Soplao (Cantabria, España). *Macla* 11, 123–124.
- Miller, C.N., 1999. Implications of fossil conifers for the phylogenetic relationships of living families. *The Botanical Review* 65, 239–277.
- Najarro, M., Peñalver, E., Rosales, I., Pérez de la Fuente, R., Daviero-Gomez, V., Gomez, B., Delclós, X., 2009. Unusual concentration of Early Albian arthropod-bearing amber in the Basque-Cantabrian Basin (El Soplao, Cantabria, Spain): palaeoenvironmental and palaeobiological implications. *Geologica Acta* 7, 363–387.
- Néraudeau, D., Perrichot, V., Colin, J.-P., Girard, V., Gomez, B., Guillocheau, F., Masure, E., Peyrot, D., Tostain, F., Videt, B., Vullo, R., 2008. A new amber deposit from the Cretaceous (uppermost Albian–lowermost Cenomanian) of southwestern France. *Cretaceous Research* 29, 925–929.
- Nguyen Tu, T.T., Derenne, S., Largeau, C., Pons, D., Broutin, J., Mariotti, A., Bocherens, H., 2000a. Lipids from fossil plants and their relation to modern plants. Examples of Cenomanian flora from Anjou and Bohemia. *Journal de la Société Biologique* 194, 57–64.
- Nguyen Tu, T.T., Derenne, S., Largeau, C., Mariotti, A., Bocherens, H., Pons, D., 2000b. Effects of fungal infection on lipid extract composition of higher plant remains: comparison of shoots of a Cenomanian conifer, uninfected and infected by extinct fungi. *Organic Geochemistry* 31, 1743–1754.
- Otto, A., Simoneit, B.R.T., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeititz Formation, Saxony, Germany. *Geochimica et Cosmochimica Acta* 65, 3505–3527.
- Otto, A., Simoneit, B.R.T., 2002. Biomarkers of Holocene buried conifer logs from Bella Coola and north Vancouver, British Columbia, Canada. *Organic Geochemistry* 33, 124–1251.
- Otto, A., Wilde, V., 2001. Sesqui-, di- and triterpenoids as chemosystematic markers in extant conifers – a review. *The Botanical Review* 67, 141–238.
- Otto, A., Walther, H., Püttmann, W., 1997. Sesqui- and diterpenoid biomarkers preserved in Taxodium-rich Oligocene lake clays, Weissenlöhle basin, Germany. *Organic Geochemistry* 26, 105–115.
- Otto, A., Simoneit, B.R.T., Wilde, V., Kunzmann, L., Püttmann, W., 2002. Terpenoid composition of three fossil resins from Cretaceous and Tertiary conifers. *Reviews on Palaeobotany and Palynology* 120, 203–215.
- Otto, A., Simoneit, B.R.T., Wilde, V., 2007. Terpenoids as chemosystematic markers in selected fossil and extant species of pine (*Pinus*, Pinaceae). *Botanical Journal of the Linnean Society* 154, 129–140.
- Pereira, R., Carvalho, I.S., Simoneit, B.R.T., Azevedo, D.A., 2009. Molecular composition and chemosystematic aspects of Cretaceous amber from the Amazonas, Araripe and Recôncavo basins, Brazil. *Organic Geochemistry* 40, 863–875.
- Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. *The Biomarker Guide. Biomarkers and Isotopes in Petroleum Exploration and Earth History*, second ed., vol. 2 Cambridge University Press, Cambridge.
- Poinar Jr., G., Lambert, J.B., Wu, Y., 2004. NMR analysis of amber in the Zubair Formation, Khafji oilfield (Saudi Arabia-Kuwait): Coals as an oil source rock? *Journal of Petroleum Geology* 27, 207–209.
- Rat, P., 1988. The Basque-Cantabrian basin between the Iberian and European plates: some facts but still many problems. *Revista de la Sociedad Geologica de España* 1, 327–348.
- Roghi, G., Ragazzi, E., Gianolla, P., 2006. Triassic amber from Southern Alps (Italy). *Palaios* 21, 143–154.
- Seoane, L.V., 1998. Comparative study of extant and fossil conifer leaves from the Baqueró Formation (Lower Cretaceous), Santa Cruz Province, Argentina. *Review of Palaeobotany and Palynology* 99, 247–263.
- Simoneit, B.R.T., 1986. Cyclic terpenoids of the geosphere. In: Johns, R.B. (Ed.), *Biological Markers in the Sedimentary Record*. Elsevier Science Publishers, Amsterdam, pp. 43–99.
- Sonibare, O.O., Ekweozor, C.M., 2004. Identification of bicyclic sesquiterpanes in oils from the Niger delta, Nigeria. *Journal of Applied Sciences* 4, 508–512.
- Soto, R., Casas-Sainz, A.M., Villalain, J., Oliva-Urcía, B., 2007. Mesozoic extension in the Basque Cantabrian basin (N Spain): Contributions from AMS and brittle mesostructures. *Tectonophysics* 445, 373–394.
- Stefanova, M., Simoneit, B.R.T., 2008. Polar aromatic biomarkers of Miocene-aged Churukovo resinite and correlation with a progenitor macrofossil. *International Journal of Coal Geology* 75, 166–174.
- Stefanova, M., Oros, D.R., Otto, A., Simoneit, B.R.T., 2002. Polar aromatic biomarkers in the Miocene Maritza-East lignite, Bulgaria. *Organic Geochemistry* 33, 1079–1091.
- Van den Berg, K.J., Boon, J.J., Pastorova, I., Spetter, L.F.M., 2000. Mass spectrometric methodology for the analysis of highly oxidized diterpenoid acids in Old Master paintings. *Journal of Mass Spectrometry* 35, 512–533.
- Villanueva-García, M., Martínez-Richa, A., Robles, J., 2005. Assignment of vibrational spectra of labdatriene derivatives and ambers: a combined experimental and density functional theoretical study. *Arkivoc* 2005 (iv), 449–458.
- Yamamoto, S., Otto, A., Krumbiegel, G., Simoneit, B.R.T., 2006. The natural product biomarkers in succinite, gessite and stantienite ambers from Bitterfeld, Germany. *Review of Palaeobotany and Palynology* 140, 27–49.