Organic Geochemistry 93 (2016) 7–21

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

# The molecular composition of Cretaceous ambers: Identification and chemosystematic relevance of 1,6-dimethyl-5-alkyltetralins and related bisnorlabdane biomarkers

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ARTICLE INFO

*Article history:*
Received 6 November 2015
Received in revised form 22 December 2015
Accepted 23 December 2015
Available online 2 January 2016

*Keywords:*
Amber
Fossil resin
Tetralin
Cretaceous
Basque-Cantabrian Basin
Labdane biomarkers

ABSTRACT

One of the major components of the organic solvent extractable fraction of Cretaceous ambers is 1,6-dimethyl-5-isopentyltetralin, a compound that has been incorrectly or not identified in previous reports on the molecular composition of Cretaceous ambers. Here, the purification and structural identification of 1,6-dimethyl-5-isopentyltetralin is reported from amber samples of the Cretaceous Peñacerrada II deposit at Peñacerrada, Álava (Basque-Cantabrian Basin, Spain). The structural identification of 1,6-dimethyl-5-isopentyltetralin allowed the complete identification of related compounds, comprising the family of 1,6-dimethyl-5-alkyltetralins. This suggests that those compounds, with concentrations up to 27% of the total organic extract of amber from Peñacerrada, are characteristic for labdane-type biomarkers in Cretaceous fossil resins and their distributions indicate different compositional families, related to their botanical and topographic origin. Also, the name amberene is proposed for the main molecule of this group. The relative proportion of the amberene-type compounds suggests three compositional families for the Cretaceous Spanish ambers.



## 1. Introduction

Amber is fossilized resin produced from the exudates of conifers and certain angiosperms (Anderson and Crelling, 1995). Analysis of the chemical composition of the organic solvent extractable fraction of fossil resins reveals a complex mixture, where the original biochemical structures of the resin compounds were modified during burial diagenesis, with the bioterpenoids (unaltered biosynthetic natural products) transformed into geoterpenoids (diagenetic products of degraded bioterpenoids that are found in amber and fossil plant tissues; Otto et al., 2002a, 2007). Despite these diagenetic alterations, geoterpenoids retain the basic skeletal structures of their biological precursors and can be used as molecular markers (biomarkers; Simoneit et al., 1986; Peters et al., 2003; Marynowski et al., 2007). Conifers synthesize mainly diterpenoids, which are, along with sesquiterpenoids, the compounds that provide diagnostic biomarkers of conifers and their resins (Otto and Wilde, 2001). The natural product diterpenoids are the main biological contributors to the extractable organic matter in amber, as a mixture of mono-, sesqui- and diterpenes, dominated by bicyclic and tricyclic hydrocarbons with a minor proportion of resin acids and alcohols preserved as such or slightly altered.

The analysis of the molecular composition of extracts from Cretaceous amber in the deposits of the Basque-Cantabrian Basin (Spain) and from the Brazilian Amazonas, Araripe and Recôncavo basins (Pereira et al., 2009; Menor-Salván et al., 2010), showed that one of the components present at high concentrations was 2,5,8-trimethyl-1-butyltetralin, a hypothetical diagenetic product from labdenoic acids. Although not identified, this hydrocarbon was also present in an extract from Peñacerrada amber (Álava, Spain; Alonso et al., 2000) and is probably a common and characteristic component in Cretaceous ambers. Despite its importance as one of the major compounds of such ambers, the identification as 2,5,8-trimethyl-1-butyltetralin was based only on interpretation of its mass spectrum and lacked a confirmed structure. We undertook the structural study of this hydrocarbon and found that the previous identification reported was erroneous. The correct structure is 1,6-dimethyl-5-isopentyltetralin (or 15,19,20-trisnorlabda-5,7,9(10)-triene, structure **I**, see Appendix), for which we suggest the name amberene due to its prevalence in ambers and because it is one of the principal

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http://dx.doi.org/10.1016/j.orggeochem.2015.12.010
0146-6380/

components of the extractable fraction. The identification of amberene allowed us to determine two additional unidentified molecules present in amber samples, that belong to the same structural family: 1,5,6-trimethyltetralin (**II**) and 1,6-dimethyl-5-(3′-methylpentyl) tetralin (homoamberene, **III**). Here, we report the purification and structural study of amberene with other related neutral and polar terpenoids in one Cretaceous amber sample. A survey of its occurrence in other Cretaceous ambers is also provided. Amberene, as the lead molecule of a novel family of 1,6-dimethyl-5-alkyltetralin biomarkers, and its related diagenetic pathways and products in ambers are also discussed.

## 2. Experimental

### 2.1. Amber collection

The materials analyzed correspond to the collection of the Museo de Ciencias Naturales de Álava (MCNA) from excavations performed between 2000 and 2010, except in the case of the Cantabria (Spain) ambers from El Soplao, Fonfría, Cuchía, Oreña and Reocin deposits and the Soto del Real (Madrid) amber, collected in the field by the authors in the Albian outcrops of El Soplao-Rabago (Cantabria, Spain; Najarro et al., 2010), Cuchía quarry (Fernández-Mendiola et al., 2015) and Fonfría, the Cenomanian outcrop of Oreña and the Reocín open pit, and the Turonian outcrop of Soto del Real (Madrid, Spain; Guidi et al., 2005).

### 2.2. Geological background

The samples used for purification and analysis of amberene belong to the Peñacerrada II amber deposit (Álava, Spain), due to the relative high proportions of amberene in the total extracts. The deposit is located in a Cretaceous succession at the Sierra Cantabria range, in the southernmost margin of the Basque-Cantabrian Basin, at the western end of the Pyrenees in northern Spain (Martínez-Torres et al., 2010). The amber was found in an Upper Aptian–Lower Albian (Lower Cretaceous) stratigraphic unit, located in the northern side of the Sierra Cantabria range (Fig. 1). The Sierra Cantabria lies over the Tertiary sediments of the Ebro Basin, as a 20 km long south-verging back thrust of Cenozoic age. The amber deposit is located in the limit between the northern limb of the thrust front and the southern limit of the Peñacerrada diapir. The deposit is hosted by a detrital unit composed of siliciclastic deposits generated in a lower deltaic plain environment and included within the Escucha formation (formerly Nograro formation), a transitional marine-continental siliciclastic sequence that reflects the transition to the continental facies of the Utrillas formation (Alonso et al., 2000; Martínez-Torres et al., 2003, 2010). The highest concentration of amber was found in the middle zone of the unit, the uppermost part of a bay-fill sequence. The paleomagnetic study of this unit suggests an Aptian (Lower Cretaceous) age (Larrasoaña et al., 2003). The subsidence modeling and the thermal history of the Escucha formation suggest a maximum temperature of 51.8 °C at the footwall of the formation, reached during the Maastrichtian (65 My), prior to maximum burial at 53 Ma (Sangüesa and Arostegui, 2003). The clay mineralogy of the host rock showed that the amber bearing sediments are composed mainly of allochthonous illite and kaolinite, transported from Hesperian source rocks in warm and wet climatological conditions. The mild diagenesis of the amber level did not alter the original clay mineralogy of the sediments (Arostegui and Sangüesa, 2003). The amber is accompanied by abundant organic matter of terrestrial/continental origin and humotelinitic meso- and macrofossil plant remains, with excellent preservation of the botanical structures (Suarez Ruiz, 2003).

Although amberene (**I**) is present in almost all the Cretaceous ambers analyzed (except for the Turonian Soto del Real amber), the Peñacerrada II deposit sample was selected due to its unusually high concentration of **I** (mean 23.0% of total organic extract, see Section 3.2). The samples composited were from transparent reddish yellow amber pieces. The crust and debris were removed and pieces inspected to avoid major inclusions.

### 2.3. Isolation of amberene

For the purification of amberene (**I**), 5 g of amber was crushed and extracted for 4 hours with dichloromethane:methanol (DCM: MeOH; 2:1, v/v) using a Büchi model B-811 automatic extractor. One aliquot of the extract was injected directly into the injection port of the gas chromatograph to obtain the molecular composition of the bulk extract. The extract was concentrated to 20 ml and fractionated using flash chromatography on silica gel. Successive elution was performed using *n*-hexane, *n*-hexane:DCM (3:1, v/v), *n*-hexane: DCM (1:1, v/v), DCM, and DCM:MeOH (1:1, v/v), and 50 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 gas chromatography–mass spectrometry (GC–MS). The fractions containing polar compounds were evaporated to dryness under $N_2$ and alcohols and acids converted to trimethylsilyl derivatives by reaction with N,O-*bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 65 °C for 3 h. The derivatized fractions were dried, dissolved with *n*-hexane and analyzed by GC–MS. This method provides a complete and representative sample of the extractable fraction of amber, allowing the study of the distribution of biomarkers in amber samples from different deposits.

The fractions enriched in amberene, and free of polar compounds, were recombined. Semi-preparative reversed phase HPLC was used to separate the concentrate and purify the amberene. A Kromasil 100 $C_{18}$ column, 5 μm, 250 × 10 mm was used with acetonitrile:water (80:20, v/v) at 3.55 ml/min. After injection of 50 μl of concentrate, separation was performed at a linear gradient from acetonitrile:water (80:20, 0 min) to 100% acetonitrile (20 min), and the chromatogram recorded by absorbance at 266 nm. Amberene was obtained (86% purity; GC–MS full scan) by collection of the peak at retention time 24.8 min. Further purification was performed by preparative thin layer chromatography using silica gel coated plates. The amberene was recovered at $R_f$ = 0.7 upon eluting with *n*-hexane:DCM (8:1, v/v). Pure amberene is a pale yellow oil with a characteristic Diesel fuel odor.

### 2.4. GC–MS

GC–MS analyses were performed with 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) and on a DB-17 column coated with (50%-phenyl)-methylpolysiloxane. The operating conditions were as follows: 8 psi He carrier gas pressure, initial temperature hold at 40 °C for 1.5 min, increased from 40 to 150 °C at a rate of 15 °C/min, held for 2 min, increased from 150 to 255 °C at a rate of 5 °C/min, held isothermal 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 the Agilent Chemstation software, and percentages calculated by normalization of peak areas of the corresponding compounds in the total extracts.