

## TERPENOID COMPOSITION OF CRETACEOUS AMBERS FROM AMAZONAS, ARARIPE AND RECÔNCAVO BASINS, BRAZIL

Ricardo Pereira<sup>1</sup>, Ismar de Souza Carvalho<sup>1</sup>, Débora de Almeida Azevedo<sup>2</sup>

1. Universidade Federal do Rio de Janeiro, Instituto de Geociências, Departamento de Geologia. CCMN, Ilha do Fundão, 21910-200, Rio de Janeiro-RJ, Brazil. ricardo.ambar@gmail.com; ismar@geologia.ufrj.br

2. Universidade Federal do Rio de Janeiro, Instituto de Química, Departamento de Química Orgânica. CT, Bl A, S/603, Ilha do Fundão, 21910-200, Rio de Janeiro-RJ, Brazil. debora@iq.ufrj.br

### Introduction

Terpenoids are common constituents of the resins higher plants and they are useful chemosystematic characteristics of extant plants, especially conifers (Langenheim, 1969; Otto and Wilde, 2001). The chemical composition of many fossil resins and ambers have been analyzed and the identified compounds were used for a chemosystematic evaluation of the source plants (Otto et al., 2002). Since many ambers are preserved separately from remains of their source plant, it is difficult to determine the botanical source based on the chemical characteristics of the amber alone. However, the fossil conifers (pollens, wood, leaves) preserved in the same lithostratigraphic units where the amber was collected may furnished indication about the amber botanic origin. Most of the compounds identified in the fossil resins are the diagenetic products (biomarkers) of terpenoids which were synthesized by living organisms (Simoneit, 1986). Despite various chemical alterations during diagenesis, the biomarkers still have the characteristic basic skeletal structures of their precursors and can thus be used as chemosystematic markers.

The chemical composition of amber is important to Paleobotany as allow to track the resin origin, and its history through the geological time. Thus, the objective of this work was the chemical analysis of Cretaceous ambers from Amazonas, Araripe and Recôncavo Basins and also the search for palinological inclusions, because this investigation opens a new search field in the Paleopalynologie.

### Samples and Methods

Three ambers from Brazilian Cretaceous basins were collected. Amazonas Basin (Alter do Chão Formation) sample was deposited in fluvial sediments of the Lower Cretaceous from Albian. The samples from Araripe (Santana Formation, Crato Member) and Recôncavo (Maracangalha Formation, Caruaçu Member) basins were deposited in lacustrine sediments of the Lower Cretaceous, from Aptian.

The samples were extracted three times for 5 min with dichlorometane/methanol in an ultra-som. The extract was concentrated and aliquots of the total extracts were converted to trimethylsilyl derivatives by reaction with BSTFA.

Gas chromatography-mass spectrometry (GC-MS) analyses of total extracts were performed on an Agilent Technologies HP5890 coupled to a HP5972 mass selective detector, using a fused silica capillary column coated with DB5. The GC operating conditions were as follow: from 60°C to 300°C at a rate of 6°C min<sup>-1</sup>. Helium was used as carrier gas. The samples was injected in splitless with the injector temperature at 290°C. Electron impact mode at 70 eV ionization energy was used. Individual compounds were identified by comparison of mass spectra with literature and library data and interpretation of mass spectrometric fragmentation patterns.

### Results and Discussion

The total extracts of the ambers contain *n*-alkanes, phenols and carboxylic acids, alkylbenzenes, alkylnapthalenes, alkylnapthalenes, and terpenoids.

Vanilic acid, vanillin, benzoic acid and isoeugenol were identified in the ambers and are interpreted as lignin products. Lignin and the phenolic derivatives are widely distributed in higher plants and thus of limited chemosystematic value. Calamene, a sesquiterpenoid of the cadinane-class, have been detected in the

ambers from Araripe and Recôncavo basins. Precursors of these sesquiterpenoid are widely distributed among the higher plants and are therefore nonspecific biomarkers (Otto and Simoneit, 2001). Alkylbenzenes, alkylnapthalenes and alkylhydronapthalenes, detected in all samples, are highly degraded diagenetic products of various sesqui- and diterpenoids. These degradation products thus cannot be assigned to certain terpenoid classes, because the basic structure of their parent molecules has been severely altered by oxidation during diagenesis.

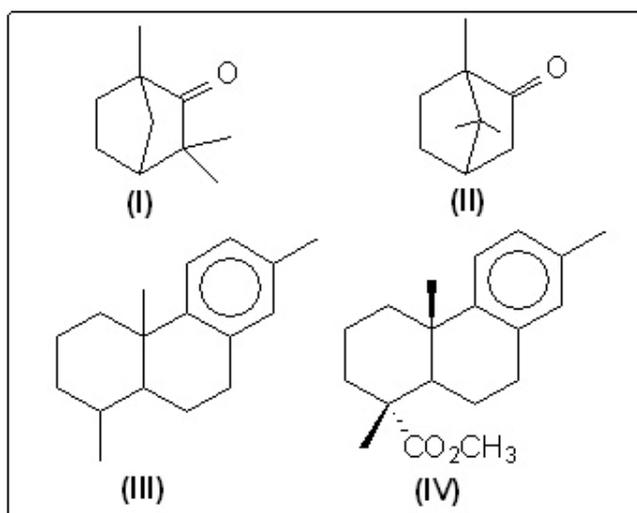
Natural products known from modern conifers, especially from the Cupressaceae and Podocarpaceae (Otto et al., 2002), like ferruginol, hydroxiferruginols, hinokiol and sugiol, were not detected in the studied amber samples under this study. Abietic acid is the major resin compound present in species of the Pinaceae family (Otto and Simoneit, 2001). Abietic acid was not detected in the ambers under study, thus these samples did not come from Pinaceae family.

Araucariaceae is suggested as the source for these ambers. This interpretation was based on the previous reported detection of araucarian fossils (pollens, wood, leaves) in the Amazonas and Araripe basins (e.g. Arai and Machado Coelho, 2001), and on the chemical similarity of the ambers with others compounds derived from Araucariaceae sources. Grimalt et al. (1988) performed a GC/MS analyses on a series of ambers and resins derived from different locations and indicated a botanical affinity with Araucariaceae. Methyl 13-isopropyl-5 $\alpha$ -podocarpa-6,8,11,13-tetraen-16-oate, methyl dehydroabietate, methyl 7-oxodehydroabietate and communic acid are important constituents of the bleed resins of Araucariaceae (Grimalt *et al.*, 1988). These compounds have not been detected in the amber samples under study. However, others important ones were detected as 7-oxo-16,17,18-trisnorabieta-8,11,13-triene, isopimara-8,15-dien-18-oic acid, 16,17-bisnordehydroabietic acid, isopimar-7-en-18-oic acid, abietatrienone, methyl dehydroisopimarate and methyl dehydroabietate. Their presences in the samples reinforce the Araucariaceae origin. Previous study on Cretaceous Brazilian amber samples (Carvalho, 1998) also suggested Araucariaceae as the botanical origin, being camphor (I), fenchone (II), methyl 16,17-bisnordehydroabietate (III) and 16,17,19-trisnorabieta-8,11,13-triene (IV) detected. These later compounds were also detected in the present Cretaceous Brazilian amber samples. Their chemical structures are presented in Figure 1.

It is noteworthy of mention that preserved fungi spores were detected in the Amazon amber sample only. It is the first reported organic inclusion in Brazilian ambers. However, Martill et al. (2005) reported in ambers from Araripe Basin micro-inclusions of possible fungal hyphae. Of note is the occurrence of this ambers in the Southern Hemisphere. Grimaldi *et al.* (2002) pointed out that most major occurrences of Cretaceous amber are know from the Northern Hemisphere. The amber localities described here, although not representing a major occurrence, are a rare and important examples of Cretaceous amber in Gondwana.

## Conclusions

The chemical composition of amber is important to Paleobotany as allow to track the resin origin, and its history through the geological time. Cretaceous amber from Amazonas Basin (Alter do Chão Formation), Araripe Basin (Santana Formation, Crato Member) and Recôncavo Basin (Maracangalha Formation, Caruaçu Member) were chemically analysed and observed through thin sections to observe probable palinological inclusions. The gas chromatography-mass spectrometry (GC/MS) analysis from the extracts identified alkylbenzenes, alkylnapthalenes, alkylhydronapthalenes, parafins, phenols, carboxylic acids and terpenoids. The presence of fenchone, camphor, 16, 17, 19-trisnorabieta-8, 11, 13-triene and methyl 16, 17-bisnordehydroabietate, besides paleobotany data from the lithostratigraphic units where the amber were collected, allowed to attribute to Araucariaceae the probable botanic origin to the Lower Cretaceous amber of Amazonas, Araripe and Recôncavo basins. In the sample from Amazonas Basin it was also identified fungi spores immersed in the fossil resin, a rare inclusion for ambers worldwide, not previously described in Brazil.



**Figure 1.** Chemical structures of selected terpenes identified in the amber samples

### Acknowledgements

R. Pereira acknowledges CAPES for the master scholarship. Financial support from CNPq and FAPERJ is gratefully acknowledged.

### References

- Arai, M., Machado Coelho, P.S., 2001. Statistical analysis of palynological assemblages from the Aptian-Albian of the Araripe Basin, northeast Brazil: a case study of paleoenvironmental significance of Early Cretaceous terrestrial palynomorphs. In: International Symposium on Mesozoic Terrestrial Ecosystems, 7, 2001. Asociación Paleontológica Argentina, Publicación Especial 7, Buenos Aires, p. 25-28.
- Carvalho, M.A., 1998. *Âmbar: Composição Molecular de Amostras Brasileiras*. MSc. Thesis, Instituto de Química, Universidade Federal do Rio de Janeiro, 108 p.
- Grimaldi, D.A., Engel, M.S., Nascimbene, P.C., 2002. Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates* 3361, 1-77.
- Grimalt, J.O., Simoneit, B.R.T., Hatcher, P.G., Nissembaum, A., 1988. The molecular composition of ambers. *Organic Geochemistry* 13, 677-690.
- Langenheim, J.H., 1969. Amber: a botanical inquiry. *Science* 163, 1157-1169.
- Martill, D.M., Loveridge, R.F., Andrade, J.A.F.G., Cardoso, A.H., 2005. An unusual occurrence of amber in laminated limestones: the Crato Formation Lagerstätte (Early Cretaceous) of Brazil. *Palaeontology* 48, 1399-1408.
- Otto, A., Simoneit, B.R.T., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz Formation, Saxony, Germany. *Geochimica et Cosmochimica Acta* 65(20), 3505-3527.
- Otto, A.; Simoneit, B.R.T.; Wilde, V.; Kunzmann, L.; Püttmann, W., 2002. Terpenoid composition of three fossil resins from Cretaceous and Tertiary conifers. *Review of Palaeobotany & Palynology* 120, 203-215.
- Otto, A.; Wilde, V., 2001. Sesqui-, di-, and triterpenoids as chemosystematic markers in extant conifers – a review. *Botanical Review* 67, 141-238.
- Simoneit, B.R.T., 1986. Cyclic terpenoids of the geosphere. In: Johns, R.B. (Ed.), *Biological Markers in the Sedimentary Record*. Elsevier, Oxford, pp. 43-99.