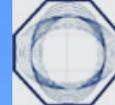


Analytical methods to differentiate Romanian amber and Baltic amber for archaeological applications



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Introduction

Amber, a fossilized natural product, is an organic material of considerable interest with respect to various scientific disciplines and art.

There are many amber species and varieties; the most well known species are the Baltic amber, the Dominican amber, the Sicilian amber named *simetit*, *brumite*-a resin extracted in the superior Burma and processed in China and the amber from Romanian Carpathians named Rumanite or Romanite.

Aim

The aim of this work was to establish several definite criteria which will differentiate Romanian amber and Baltic amber to certify the local or Baltic origin of the materials found in archaeological sites on the Romanian territory, performing analytical methods, such FTIR and HPLC with MS detection.

Materials and Methods

• **Light microscopy** in the study of fossil resins has a main importance. Apart from efficiency and easiness, it offers a precision grade comparable only to the chemical analyses and it allows detailed mineralogical investigations of the resins and the host-rock. Samples of Baltic amber and Romanite were studied as thin sections by transmitted light and microscopic techniques used in petrography. A Pamphot Microscope equipped with an automatic Nikon Eclipse camera E-400, 40 W was used.

• **HPLC-MS method** was developed to assess the succinic acid amount (specific hydro soluble component in the amber structure). The extraction was performed using a polar solvent, acetonitrile in acid media (pH = 3.00). Samples were grounded and extracted in acetonitrile acidified with formic acid (pH = 3.00), 1:1 ratio (mg/mL) at room temperature, 7 days long. We should mention that samples of Romanite are easily grounded while Baltic samples are harder and difficult to ground. The extracted fractions were filtered through 22 mm Millipore filters and analysed by LC-MS. The LC-MS experiments were carried out on a Shimadzu system equipped with two pumps LC-20AD, photodiode array detector SPD-M20A (operating between 200 and 600 nm) and coupled to a MS detector Shimadzu LCMS-2010EV equipped with an ESI interface. The separation was done on a 50 x 2.1 mm Kromasil 100-3.5 C18 column, at 200C. UV and MS data were acquired and processed with a LC-MS Solutions operating system. The mobile phase used was: solvent A = 25% deionised water with formic acid, pH=3.00; solvent B = 75% acetonitrile with formic acid, pH=3.00, isocratic. The time of running was 15 min; 5 min of equilibration was required before the next injection.

• **FTIR** assay was performed using FTIR-VAR technique (Fourier Transformed Infrared Spectrometry, Variable Angle Reflectance) with a beam incidence angle of 45°, on a Bruker TENSOR 27 instrument. The samples were used without any pre-treatment, as whole pieces, all the spectra were registered versus a background on clean gold foil. All experiments were performed in inert atmosphere (purging spectral purity Nitrogen) to acquire an appropriate resolution in region characterized of high wavenumbers (3200-3700cm⁻¹) where the form and the frequency of the molecular absorption bands from this region are ascribable to the presence of carboxylic hydroxyls in free form or inter/intramolecular bound.

Results

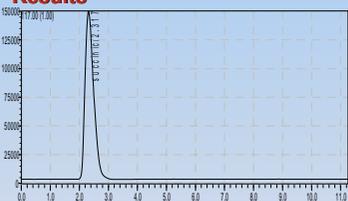


Fig.1 Chromatogram of sample 383 (Colti-Patralagele)



Fig.2 Romanian amber; Romanite (Colti) (spore inclusion, resin replace spore organic tissue)

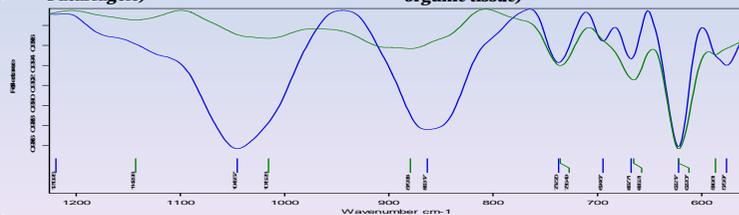


Fig.3 FTIR-VAR (45°) spectra comparison, region 650-900, for Baltic amber (211) and Romanian amber (204)

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Table1. Composition in succinic acid obtained by LC-MS from samples with controlled origin (Baltic amber and Rumanite).

No Crt	Sample ID	Succinic acid µg/mg amber	Composition (%) succinic acid
1	211/Baltic	1.04±0.20	0.1%
2	204/Rumanit-Colti	7.19±0.08	0.72%
3	209-Copalit	4.42±0.15	0.44%
4	301-Palanga Lithuania	0.43±0.03	0.04%
5	280-Colți Buzău	0.19±0.05	0.02%
6	321-Colți Pătărlagele-gallery	6.77±0.06	0.67%
7	306-Sibiciul de jos	1.45±0.11	0.14%
8	329-Poland	1.11±0.08	0.11%
9	341-Kaliningrad	0.27±0.05	0.027%
10	355-Bitterfeld Germany	0.82±0.12	0.08%
11	383-Colți Pătărlagele	7.75±0.03	0.77%
12	119-Colți	1.66±0.01	0.16%

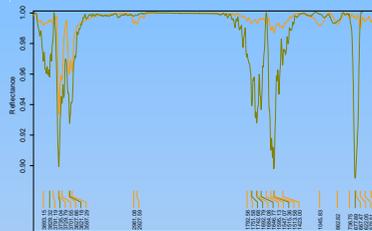


Fig.4 FTIR spectra for samples 321 and 204 (Romanian amber samples)

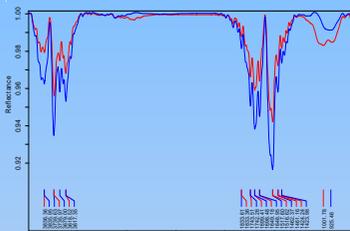


Fig. 5 FTIR spectra for samples 341 and 345 (Baltic amber samples)

Conclusions

► **Light Microscopy analysis** demonstrate that diagenetic processes played a significant role in the Romanian creation: it's characteristic small surface cracks as a consequence of the lessening of free water proportion confirm this fact while in comparison the Baltic amber analysis proved a lot of fluid inclusions. It could see also remineralisations in Romanite, e.g. substitution of organic matter by anhydrite and feldspar. In other situation, the organic tissue was replaced by resin.

► The concentration of succinic acid was determined by LC-MS method described above using a calibration curve ($Y = 284940.0X - 108595.5$; $R^2 = 0.9870$; $R = 0.9935$). The linear domain of succinic acid concentration levels (Aldrich) was ranging 0.1 and 10 µg/mL. The obtained detection limit was 0.085 µg/mL. The MS detection is very sensitive, 500 times more sensitive than UV detection. The retention time was the same for standard and samples. The quantitative results for succinic acid found in different samples (searched ion mass = 117) are presented in table 1.

The results obtained by LC-MS gave results of concentrations ranging between 0.27 and 1.11 µg/mg of succinic acid in Baltic samples and concentrations between 1.66 and 7.75 µg/mg succinic acid in Romanite samples. Therefore, we could conclude that it is a possibility to make a distinction Romanite from Baltic using the amount of succinic acid determined by this technique, the quantities of succinic acid extracted in described conditions being higher for Romanite samples (and for other species of younger resins, such as copal). The explanation could be the fact that, in applied extraction conditions, succinic acid is extracted easier from lax structures, and the extraction is more difficult from dense structures (like Baltic amber structure, older and with more bonds which must be unbound).

It must be emphasized that it was not performed a total extraction of the succinic acid from samples by this method, therefore our results are not comparable with those published in literature about the different ratios of succinic acid from different types of amber. In addition, the difference could appear because amber is not a homogenous material. Still, the results are interesting, and LC-MS analysis together with other methods, could bring a certain decision about the origin of material.

► FTIR-VAR spectra were analysed and explained on the three wavenumbers domains of interest for amber, namely 2000-3600 cm⁻¹ zone, 1350-1820 cm⁻¹ zone and 1045-1250 cm⁻¹ zone, which correlate with hydroxyl groups, carbonyl groups, carbonyl groups and with C=C unsaturation.

As a result of this observations, for each sample were registered two spectra, for different zones of sample, in order to notice if significant differences appear between the two spectra of same sample, due to appearance/disappearance of certain absorption bands (specific vibration frequencies of interest). It was observed that difference between two spectra of same sample appear only in spectral region related to -CH₃, -(CH₂)n-, -CH, chains, namely 2962 – 2850 cm⁻¹, and for the specific tensile bands of CH₃ from 1375cm⁻¹. That means that the presence of functional groups, correlated with origin and the age of sample (specificity of sample) are not unequally distributed, therefore the analytic information supplied by FTIR is acceptable.