Preliminary characterization of Palaeogene European ambers using ToF-SIMS

Rana N. S. Sodhi,a* Charles A. Mims,a Robyn E. Goacher,a Bruce McKaguea and Alexander P. Wolfeb

Introduction

Amber comprises polymerized plant resins that have a remarkable capacity for survival in a range of geological environments. Most ambers can be traced to coniferous trees and typically combine a broad array of plant natural products including terpenoids, carboxylic acids, and associated alcohols. Because amber may entomb various organisms at the time of production and preserve them with unmatched fidelity, it has been studied for centuries. Despite extensive geochemical profiling of amber-derived extracts using techniques such as gas-chromatography mass spectrometry, to date amber compositional variability has not been investigated by time-of-flight secondary ion mass spectrometry (ToF-SIMS). We conducted a series of scans on micromated surfaces of Baltic and Bitterfeld ambers, representing two of Europe’s major deposits, both of Palaeogene age. We exploited authentic standards of mono-methyl succinate and diterpene resin acids to guide interpretation of the results. The ToF-SIMS spectra are highly reproducible for each amber type considered and highlight subtle differences that are likely underscored by differences in age, botanical provenance, and post-depositional history. Importantly, the abundance of succinate is consistently higher in Baltic amber relative to Bitterfeld amber, suggesting they are distinct deposits and not regional variants of each other. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Baltic amber; Bitterfeld amber; succinate; diterpene; time-of-flight secondary ion mass spectrometry; principal component analysis

However, some of these differences can be very subtle, and therefore, given the myriad of information present in a typical ToF-SIMS spectrum, difficult to disentangle. To help elucidate these differences, principal component analysis (PCA) was exploited to compare three related yet lithologically distinct forms of amber from northern Europe. To the best of our knowledge, this constitutes the first application of ToF-SIMS to the study of amber.

Baltic amber is the single largest deposit of fossil conifer resin known. It represents a widely disseminated archaeological material and an important paleontological resource. Baltic amber is primarily found in sediments of Middle Eocene age (44–47 million years old), but never occurs in its original stratigraphic position: throughout its geographical range (spanning much of the Baltic and North Sea basins), it has been redeposited mainly in fine-grained marine lithologies, and sometimes in younger glacial and fluvial sediments. Glessite is a dark variant of Baltic amber that is characterized by advanced oxidative weathering of its surface. Despite being visually different, it differs little from Baltic amber with respect to overall composition. Bitterfeld amber originates from the Goitzsche mine near the town of Bitterfeld (Sachsen-Anhalt, Germany) and appears to have a more localized distribution. Relative to Baltic amber, the Bitterfeld amber is contained in significantly

* Correspondence to: Rana N. S. Sodhi, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON M5S 3E5, Canada. E-mail: rns.sodhi@utoronto.ca

a Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, M5S 3E5, Canada

b Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton AB T6G 2E3, Canada

younger marine sediments (Latest Oligocene, 24–25 million years old), suggesting that the amber is considerably younger and hence a geologically distinct deposit. However, the recognized similarity between insect and spider assemblages from both ambers makes it possible that Bitterfeld amber represents secondary redeposition of Baltic amber, and that the ambers themselves are in fact coeval to each other. This debate remains unresolved, and serves as an impetus for the current investigation.

Baltic amber, glessite, and Bitterfeld amber are all representatives of Class IA fossil resins in the classification scheme of Anderson et al., meaning that they are dominantly polymerized labdanoid diterpenes, typically including comminic acid and communol, and further characterized by the incorporation of significant amounts of succinic acid. Indeed, succinic acid and other succinates are defining biomarkers for Baltic amber and the reason that it is sometimes referred to as succinite. In this study, we compared ToF-SIMS spectra obtained from representative samples of these three ambers. Our general objective is to provide an initial evaluation of the promise of ToF-SIMS in the study of amber. Our more specific objective is to explore whether or not Baltic and Bitterfeld ambers are coeval to each other and derived from the same original forests.

Materials and methods

Representative samples of Baltic amber and glessite (both from Kämpinge, Skåne, Sweden) and of Bitterfeld amber (from Goitzsche) were adhered to standard SEM stubs and cut with a diamond blade using a Leica EM UC6 ultracryomicrotome (Leica Microsystems GmbH, Vienna, Austria). This was done immediately prior to introducing the sample into the ToF-SIMS load lock; no further sample preparation was performed. Other pretreatments were initially evaluated, including polishing with diamond foil using a Leica EM TXP Target Sectioning System. Although suitably flat surfaces were produced, inconsistent results were obtained due to surface contamination and possibly partial dissolution of organic components when surfaces were wiped clean with chloroform.

The amber spectra were compared to those obtained from representative samples of these three ambers. Our general objective is to provide an initial evaluation of the promise of ToF-SIMS in the study of amber. Our more specific objective is to explore whether or not Baltic and Bitterfeld ambers are coeval to each other and derived from the same original forests.

![Figure 1](image-url) Negative ToF-SIMS spectra for (A) Baltic amber, (B) Glessite, and (C) Bitterfeld amber. In each case, the upper row is the complete spectrum, whereas the middle row shows expansion of the 40–200 amu region, and the lower row expansion of the 200–400 amu region. The peaks marked by * represent succinate, those by # phosphate, and that by + a dominant diterpene fragmentation product.
such as ESI tandem MS have also favoured the negative polarity mode, albeit due to higher signal to noise.\(^{[15]}\) In contrast, ToF-SIMS spectra obtained in negative polarity mode appear much more interpretable in terms of both differentiating the three different ambers and identifying major peaks on the basis of results obtained from the standards. Figures 1 and 2 illustrate the negative ion mass spectra for the three ambers and standards, respectively, including in each case complete spectra as well as expansions of the 40–200 amu and 200–400 amu regions. All subsequent analyses are based on these results.

Negative ion ToF-SIMS spectra from the Class IA ambers contain a number of peaks elucidated by the standards, including succinate (117.02 amu), anhydrous succinate (99.01 amu), and a range of diterpene fragments in the 300–400 amu region, dominated by the peak at 301.21 amu which is present in both abietic and communic acids (Fig. 2). A more exhaustive consideration of diterpene ToF-SIMS results will be presented elsewhere, including a range of additional compounds. While the 301.21 amu diterpene peak is somewhat muted in the ambers relative to the standards, it is noteworthy that this peak is much more pronounced in Baltic amber and glessite relative to Bitterfeld.

Figure 2. Negative ToF-SIMS spectra and structures of the three analyzed standards: (A) mono-methyl succinate, (B) abietic acid, and (C) communic acid. Rows are displayed as in Fig. 2, as are the labeled peaks.

Figure 3. Sample scores and corresponding loadings for the first principal components extracted from centered and square-root transformed ToF-SIMS peaks for ranges 40–200 amu (A and B) and 200–700 amu (C and D).
amber. Similarly, both succinate peaks (99.01 and 117.02 amu) are also more weakly expressed in Bitterfeld amber (Fig. 1). Additional peaks of interest that are not displayed by the standards include those present at 79.97 amu and 96.97 amu and assigned to HPO₃ and H₂PO₄, respectively. These are far more strongly expressed in Bitterfeld amber relative to the two other ambers. In summary, the amber negative ion spectra reveal that the abundance of succinate is higher in Baltic amber and glessite relative to Bitterfeld amber, but that Bitterfeld amber has more phosphate.

These differences were further explored using PCA. Results for the first principal component obtained from the 40–200 amu and 200–700 amu subsets are illustrated in Fig. 3. For the broader mass range (40–700 amu), the results were similar and dominated by the intense low-mass peaks captured by the 40–200 amu model. In the PCA models of both mass ranges, the leading principal component clearly differentiates Bitterfeld amber (more negative sample scores) from Baltic amber and glessite (positive scores). This result is mainly due to the greater relative abundances of succinate-related fragments (117.02 and 99.01 amu) in Baltic amber and glessite, and the presence of more phosphate ions (79.97 and 96.97 amu) in Bitterfeld amber. In the higher molecular mass diterpene region, the strong loading of 335.24 and 351.23 amu in Baltic amber and glessite could indicate the presence of more abietic acid (or potentially other diterpenes). Hydration of the 317.21 amu peak in the abietic acid standard mass spectrum, and/or protonation of the peaks at 333.20 and 349.20 amu, may account for these PCA loading patterns. Irrespective of these details, the leading principal components extracted from both mass ranges yield remarkably concordant results, from which we surmise that Bitterfeld amber is indeed compositionally distinct from either Baltic amber or glessite. Differentiation of glessite from the other ambers was observed with the second principal component (not shown). In this case, positive loadings observed at 79.97, 99.03 as well as 63.97 amu (HPO₂) suggest subtle differences in the phosphate profile of glessite. However, because phosphorus is not a major constituent of Class IA ambers, it is likely that these differences reflect post-depositional processes that modulated the incorporation of P from sediment pore water into amber during initial polymerization. Likewise, glessite was again differentiated from the other ambers in the higher mass diterpene region, this time by a more complex suite of peaks, which is yet to be fully understood. Higher order principal components did not provide additional distinguishing features between the three sample types.

Conclusion

While these results must be considered preliminary in the sense that they are restricted to three Class IA ambers and only a small number of standards, they nonetheless demonstrate the applicability and efficiency of ToF-SIMS as a robust tool to investigate the chemistry of amber. A major advantage is that whole samples in the solid phase can be analyzed with a minimum of pretreatment, yielding reproducible, high-resolution compositional information. This overcomes problems inherent to techniques that mandate solvent extraction prior to analysis, because amber constituents are variably soluble as a function of their polymerization and cross-linking histories. For example, the Class IA ambers considered here are only partially soluble in organic solvents.

The most important result of our comparative investigation is that Bitterfeld amber consistently contains less total succinate than either typical Baltic amber or its more weathered and oxidized counterpart, glessite. This supports the notion that Bitterfeld amber is a distinct and younger deposit,[7,8] despite over-arching similarities with respect to arthropod species preserved as inclusions in these ambers. If succinic acid and associated succinate esters in Class IA ambers are considered to be products of diagenesis and not source plant metabolism, as suggested elsewhere,[2] their lower concentrations in Bitterfeld amber are entirely consistent with a younger age. If this age difference is truly in the range of 20 million years (i.e. Middle Eocene to Late Oligocene), there are important implications for the paleoentomological record. This is because the maximum estimated duration of individual insect species, presently inferred to be 3–10 million years,[16] will require substantial upward revision in order to reconcile the co-occurrent taxa in Baltic and Bitterfeld amber.

Acknowledgements

This research was supported by the Canadian Foundation for Innovation (Surface Interface Ontario), the Ontario Research Fund, and Natural Sciences and Engineering Research Council of Canada Discovery Awards to CAM and APW.

References