Amber fascinates by its color, refraction of light, and golden glow, but how can you tell if it's the real thing?

Material analysis

Amber authentication, a closer look

Amber is cherished for its beauty and is used in jewelry and works of art. Amber has been thought to have healing powers and has been widely used for charms to keep bad luck at bay. However, it is not easy to know if the gem in your hand is real amber or a well-produced fake. Looks can be deceiving and so can the texture of the surface. Age is what matters and older is better: A million years is the bare minimum - orders of magnitude more the norm. While amber doesn't easily reveal its age or origin, instrumental analysis can shed light on the matter: Pyrolysis coupled with gas chromatography (GC) and mass spectrometry (MS) is known as a strong complement to spectroscopic techniques. If you automate the pyrolysis and add a suitable reagent to perform thermochemolysis, detail on the amber's origin is revealed.

The town of Ribnitz-Damgarten lies beautifully situated where the river Recknitz flows into Lake Ribnitz in the German State of Mecklenburg-Vorpommern. The town of 15,000 inhabitants is a very special place as can be seen by visiting its homepage. Since 2009, Ribnitz-Damgarten has been officially designated as the "Amber City." The reason is the Amber Manufacture in the Damgarten section of town and the German Amber Museum in the Ribnitz section. The town boasts an amber shopping mile where amber of every shape and hue can be acquired. In addition there is the annual Amber Queen Contest; the International Amber Art Competition; and the world's longest amber necklace at 120 meters. At the Baltic Seashore not far from town, visitors can spend the day hunting for amber, the "Gold of the sea". Whether or not amber found on the beach is the real thing can be determined by a simple density test as recommended by the German Amber Museum. The following experiments will provide good indications as to the type of amber found and whether it is real:

- Produce a saturated salt solution by adding two soup spoons of salt to a glass of water.
- Amber will float in this solution, stone, glass and polymer materials will sink.
- Compare: Among the different amber

types, clear amber is the heaviest, whereas "amber bone" is the lightest.
- Rub larger amber chunks against wool. They will build up an electrostatic charge and attract paper snippets.
- Carefully tap the find against your front teeth. If it is amber, it is not unpleasant and will produce a dull sound. Amber produces the same sensation as if you tap your finger nail on the teeth. A yellow pebble or a glass shard will sound light and their contact with the teeth will produce a distinctly unpleasant sensation.

The Johannes Gutenberg University in Mainz, Southwestern Germany is far away from the Baltic coast, but even here amber is high on the agenda. Dr. Oluwadayo O. Sonibare and Prof. Dr. Thorsten Hoffmann from the Department of Inorganic and Analytical Chemistry along with Prof. Dr. Stephen F. Foley from the Dept. of Geosciences and Earth System Research Centre are searching for a more accurate method to determine origin and authenticity of amber. The recommendations from the German Amber Museum are useful for tourists and for those who casually collect amber, but of course not for university level scientific research on the matter. The stated goal of the scientists is to determine "The molecular composition and chemotaxonomy aspects of Eocene amber from the Ameki Formation in Nigeria [1], the project is sponsored with a research grant from the Alexander von Humboldt Foundation. For the geochemical characterization and biochemical classification, Sonibare and his colleagues have previously used Infrared (IR) Spectroscopy and Gas Chromatography (GC) with Mass Spectrometry (MS) detection to determine the molecular composition of different fossilized plant resins. They are now looking to automated pyrolysis as well as thermochemolysis to help move the project forward.

What is known about amber?

Amber, in mineralogical terms also known as succinite, was formed when tree resin from a fir or other coniferous species was hardened through contact with air millions of years ago. Large quantities of dried resin wound up in the oceans and sank into deep sediment layers, which were in turn covered by more layers of sand and rock. Over time and under huge pressure in an anaerobic environment, amber was formed. Most amber finds have been dated to the tertiary period, around 55 million years ago. However, "younger" species are known as well. To be classified as amber, at least a one million year maturation period is required. Baltic amber is among the most ancient of known ambers. Amber is found in many countries, for example, in England, Portugal, Spain and Italy, but also in Canada, Mexico, Japan, the Dominican Republic, in Madagascar, and in Borneo. Amber fascinates through the varying coloration, light refraction, and the golden yellow glow after polishing. Amber feels sensuous and warm, and is surprisingly light. It makes you want to touch it, have it against your skin, and use it as a hand charm, letting your fingers run over it. Even today, people on the Baltic shore actively collect amber, which is also said to have healing, pain-killing and calming properties. Sometimes, million-year-old insects or plants are clearly visible, encased and fossilized in the amber.

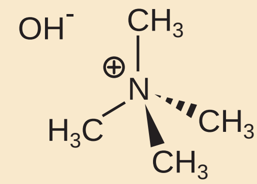
Amber, in mineralogic terms called succinite, is fossilized tree resin, which seeped from firs or other conifers millions of years ago

The remains of insects and plants that lived millions of years ago are sometimes found encased and fossilized in amber.

Delving into the details

At GERSTEL Headquarters, R&D Project Manager, Dr. Eike Kleine-Benne and his colleague Yunyun Nie met with Dr. Sonibare from the Johannes Gutenberg University to exchange information on the properties of amber with a view to determining both origin and authenticity. In short: What are the possibilities for determining the composition and specific properties of amber and thus finding markers for different types of amber? Deciding on which technique to use is easier if you have detailed knowledge of the composition of the material. According to Dr. Sonibare, the fossilization of plant resin is a complex curing and maturation process involving

$$R\text{-}COOH \xrightarrow{TMAH} [R\text{-}COO]^-[(CH_3)_4N]^+ \xrightarrow{\Delta} R\text{-}COOCH_3 + (CH_3)_3N$$



Methylation of a carboxylic acid using tetramethylammonium hydroxide (TMAH). Shown to the right: TMAH structural formula.

loss of volatile content, polymerization, and crosslinking of terpenoids over a period of up to 100 million years. Amber is sometimes considered a natural polymer, but chemically speaking ambers are heterogeneous mixtures of polyesters classified as terpenoids (mono-, sesqui-, di- and triterpenoids), all of which are derived from isoprene, a compound that occurs naturally in plants. In contrast to terpenes, terpenoids contain functional groups. The analysis techniques mainly used for the characterization of amber are infrared (IR) spectroscopy, Raman spectroscopy, GC/MS analysis of solvent-based extracts, and pyrolysis-GC/MS. Based on the chemical and physical properties of amber, the scientists decided to use automated pyrolysis-GC/MS, but also to take it to the next level: Thermochemolysis, combining pyrolysis and a derivatization reagent, in this case tetra methyl ammonium hydroxide (TMAH), a technique previously described in literature. The hope and theory was that the derivatized pyrolysis fragments could be more extensively characterized, providing deeper knowledge of the composition and enabling a more accurate classification of amber types and their geographical/geological origin.

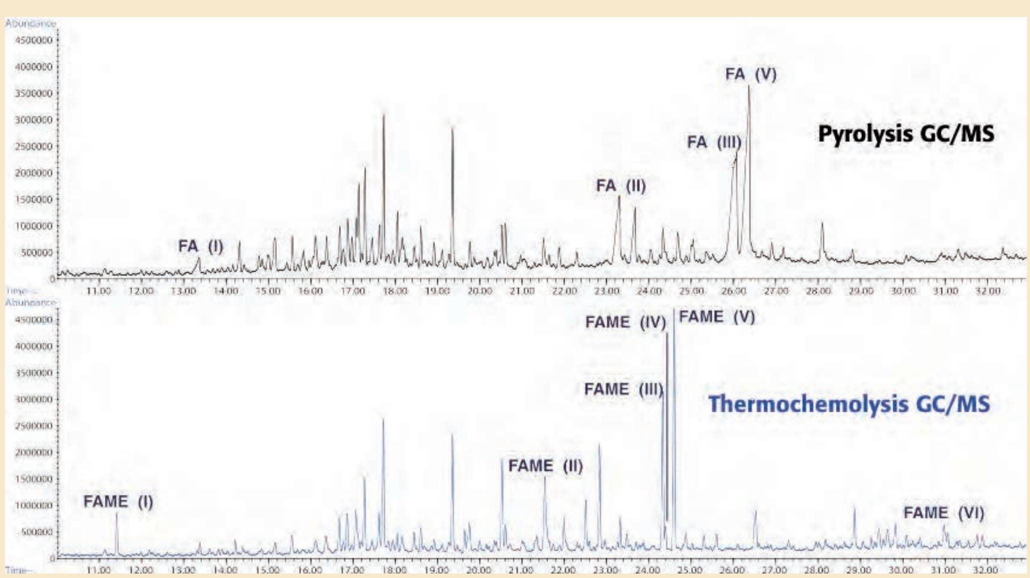
IR spectroscopy is used to differentiate Baltic amber from other ambers, says Eike Kleine-Benne. The main useful IR differentiator is the so-called "Baltic shoulder" at 1250 to 1175 cm-1. This absorption band is associated with succinic acid, which is found in Baltic amber. However, with regard to amber classification and differentiation in general, the IR method is of limited use, due to the fact that different amber types often yield similar patterns.
Raman spectroscopy is useful for the determination of differences in amber maturation processes, not for geographical origin.
GC/MS enables a molecular – structural elucidation of the soluble part of amber, which unfortunately only constitutes 20 % of the total.
Pyrolysis-GC/MS gives the analyst the possibility of generating fragments of the polymer parts, i.e. to split insoluble and non-volatile macromolecules into individual fragments, separate these and determine their identity based on a detailed mass spectrum. The fragment pattern and certain markers are then used to determine the type and origin of amber samples.
Thermochemolysis combines pyrolysis and a derivatization reagent. This means that as soon as certain pyrolysis fragments are formed, they are derivatized in order to enable or improve their determination by GC/MS.

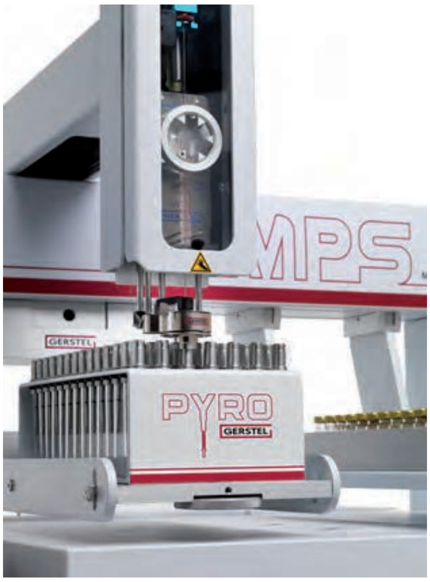
Amber is flammable, it burns generating a distinct aromatic smell and it was long used as substitute for incense. Amber shows limited solubility in alcohol, ether, chloroform, and turpentine oil. Ground amber is negatively charged and has a melting point of 375 °C.

Hunting for data and gathering information with pyrolysis GC/MS

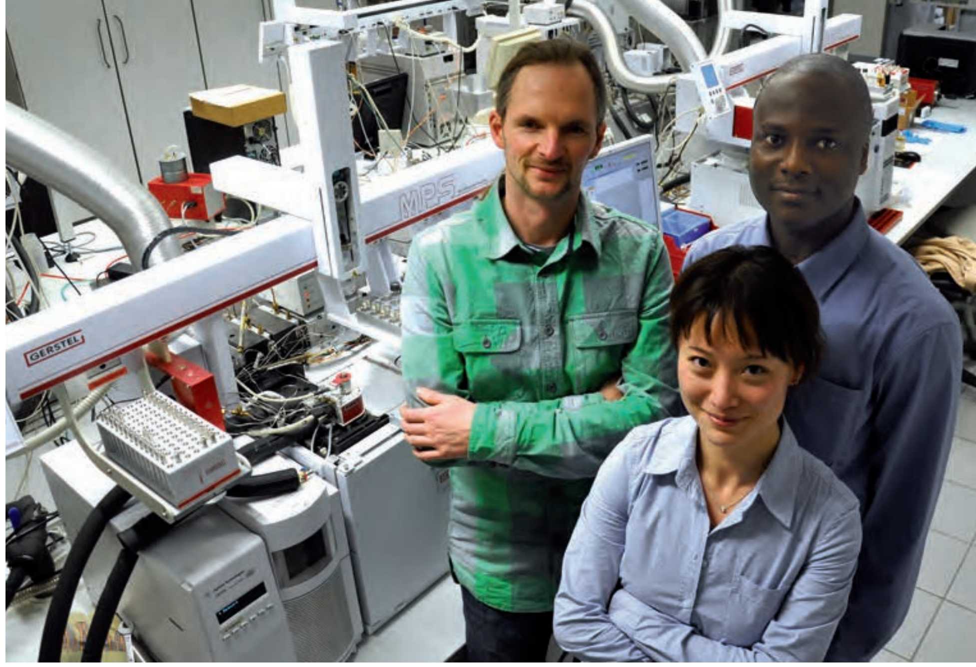
Eocene amber samples from the Ameki formation in Nigeria were kindly made available for the project by the University of Glasgow Hunterian Museum. The sample material was ground and 200 µg portions were individually pyrolyzed at 480 °C for 20 seconds. The following pyrolysis-GC/MS system was used: Agilent GC 6890N with GERSTEL Cooled Injection System (CIS), Agilent 5795B Inert

Comparison of chromatograms resulting from pyrolysis GC/MS and thermochemolysis GC/MS, respectively, of Eocene amber from Nigeria (FAME: Fatty acid methyl ester / FA: Fatty acid).





GERSTEL PYRO provides more in-depth information on the analyzed amber


The amber team from right to left: Oluwadayo O. Sonibare, Ph.D., Yunyun Nie, and Eike Kleine-Benne, Ph.D..

XL (Triple Axis) MSD, GERSTEL MultiPurpose Sampler (MPS), GERSTEL Thermal Desorption Unit (TDU), and GERSTEL PYRO pyrolysis module mounted on the TDU. PYRO is a plug-in unit, which can be mounted on the TDU in just a few minutes and allows automated sample introduction when used with the MPS. Technical detail: The TDU was operated in split mode in order to vent excess solvent and volatile organic compounds (VOCs). The CIS was set to an initial temperature of 300 °C and used as heated split interface, with a split ratio of 1:20.

The column used was a ZB-5MS fused silica capillary column (30 m x 0.25 µm x 0.25 mm) from Phenomenex. The GC oven temperature program was set as follows: 60 °C (2 min) – 6 °C/min – 300 °C (10 min) using Helium carrier gas at 1 mL/min. The MSD was operated in Electron Impact (EI) ionization mode with an ionization energy of 70 eV, and source and quadropole temperatures set to 230 °C and 150°C respectively. Full scan spectra were acquired from 50 to 650 Da.

Data acquisition and data handling were performed using ChemStation Software (Agilent Tecnologys) and compound identification performed using both the integrated mass spectral library and information from literature.

Gaining deeper knowledge step by step

Initially, a ground amber sample was pyrolyzed. In the chromatogram, a long line of the "usual suspects" were identified, which were previously described in literature. In this case, a series of fatty acids (FAs), among them:

- Norchrysanthemic acid (FA I)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl (FA II)
- Naphthalene-1-Carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl (FA III)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene (FA V).

To gain better chromatographic performance and more information, amber samples were pyrolyzed in the presence of tetramethyl ammoniumhydroxid (TMAH) for in situ derivatization (methylation) during the 20 s pyrolysis period. This process is called thermochemolysis; fatty acids are converted into their corresponding methyl esters (FAMEs):

- Norchrysanthemic acid methyl ester (FAME I)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl methyl ester (FAME II)
- Naphthalene-1-Carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl methyl ester (FAME III)
- Methyl-1,2,3,4-Tetrahydro-1,5,6-Trimethyl-1-Naphthalenecarboxylate (FAME IV)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene methyl ester (FAME V).
- Methyl-16,17-Dinorcallitrisate (FAME VI)

The difference in performance between pyrolysis and thermochemolysis is explained by Eike Kleine-Benne as follows:

„In the pyrolysis chromatogram, the acids peaks (FA (I), FA (II), FA (III) and FA (V) are broad, and partly saw tooth shaped, a typical phenomenon for highly polar or active compounds such as free fatty acids (FFAs) separated on a non-polar column. FA (IV) and FA (VI) are not found at all. In contrast, the corresponding methylated acids elute as well separated sharp peaks in the thermochemolysis-based chromatogram, including FAME (IV) and FAME (VI). It is clear that pyrolysis with TMAH brings out more detailed information about the analyzed material." As Dr. Sonibare reports, an earlier study yielded the first available information on the molecular composition of fossilized resins from the Eocene Ameki formation in Nigeria with pyrolysis GC-MS analysis clearly indicating that the amber belongs to class I b, which is derived from regular labdatriene structures. Another characteristic of class I b ambers is that they do not contain succinic acid. The pyrolysis products of the amber were dominated by labdanlike diterpenoids and a small number of sesquiterpenoids. The exclusive presence of labdane diterpenoids and the absence of plant triterpenoids in the Eocene Ameki amber pointed to a conifer (Gymnosperm) origin of the resin. With the described method, Dr. Sonibare, Prof. Hoffmann and Prof. Foley have a tool which helps them categorize amber by origin while delivering further information for their amber research: New research results were recently published in the Journal of Analytical and Applied Pyrolysis [2].

Literature

[1] O. O. Sonibare T. Hoffmann, S. F. Foley: Molecular composition and chemotaxonomic aspects of Eocene amber from the Ameki Formation, Nigeria, Organic Geochemistry 51 (2012) 55–62

[2] O. O. Sonibare, R.-J. Huang, D. E. Jacob, Y. Nie, E. Kleine-Benne, T. Hoffmann, S. F. Foley: Terpenoid composition and chemotaxonomic aspects of Miocene amber from the Koroglu Mountains, Turkey, Journal of Analytical and Applied Pyrolysis 105 (2014) 100-107