

# On the Chemistry of Baltic Amber Inclusion Droplets

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**Summary.** Baltic amber inclusion droplets were analyzed by means of gas chromatography, mass spectroscopy, FTIR spectroscopy, and capillary electrophoresis. It turned out that they were constituted of water in which a variety of inorganic cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) and anions ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) were dissolved. Moreover,  $\text{NH}_4^+$ , acetate, and succinate ions were detected with the latter strongly predominating. However, no terpenes could be traced. From these results it was concluded that the inclusion droplets stem from splash water of a saline environment into which amber genuine succinic acid was extracted. Ammonium and acetate ions could originate from either tree sap contents or biogenic contents of the splash water.

**Keywords.** Amber; Succinate; GC-MS; FTIR; Capillary electrophoresis.

## Zur Chemie von Inklusionströpfchen des baltischen Bernsteins

**Zusammenfassung.** Inklusionströpfchen des baltischen Bernsteins wurden mit Hilfe von GC-MS, FTIR Spektroskopie und Kapillarelektrophorese analysiert. Es stellte sich heraus, daß es sich dabei um Wasser handelt, in dem eine Reihe von anorganischen Kationen ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) und Anionen ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) gelöst sind. Darüber hinaus enthalten sie auch  $\text{NH}_4^+$ , Acetat- und Succinationen, wobei letztere in großem Überschuß vorhanden sind. Terpene waren jedoch nicht nachzuweisen. Aus diesen Ergebnissen wurde abgeleitet, daß die Inklusionströpfchen von salinem Spritzwasser stammen, in welches aus dem Bernstein stammende genuine Bernsteinsäure extrahiert worden war. Ammonium- und Acetationen könnten entweder aus den Inhaltsstoffen des Baumsaftes oder aus biogenen Materialien des Spritzwassers herrühren.

## Introduction

Amber, a fossilized natural product, is an organic material of considerable interest with respect to various scientific disciplines and art. Whereas paleontology focuses mainly on the wonderfully preserved inclusions of extinct plants or animals [1], molecular biology is devoted to studies of DNA remnants of organisms to derive family trees and relations between old and recent species [2]. Chemistry is mostly interested in the material itself, thus supplying information about its composition [3] and formation [4]. Moreover, it has been possible to correlate the various ambers by means of their chemical fingerprints and thus provide arguments for the provenience of amber artifacts [5].

A variety of ambers contain small to submicroscopic bubbles [1]. Most of them are empty or filled with entrapped gas, which has evoked a vivid investigation of their contents with respect to the question of an entrapped "fossilized" air [6]. In addition to these gas inclusions, tiny liquid droplets can also be occasionally found as inclusions in certain ambers. They have been speculated to contain liquid terpenes [7]. However, from the standpoint of terpene chemistry [8] one would not easily understand why a simple liquid isoprenoid compound would have survived millions of years without severe oligomerization or polymerization leading to a solid amberlike material. To solve this question, the present paper is devoted to establish a chemical inventory of such amber inclusion droplets.

### Results and Discussion

First of all, it became clear that the droplets were aqueous and did not contain organic liquids at all. This was concluded from the FTIR spectrum shown in Fig. 1. The latter contained a very intensive band system in the O–H stretching region characteristic of water. In addition, prominent bands in the C=O stretching region could be observed, pointing to the presence of carboxylate ions. It should be mentioned that the prominent band around  $2350\text{ cm}^{-1}$  was not due to the sample but stemmed from atmospheric  $\text{CO}_2$  present in the light path. Moreover, the droplets proved to be perfectly miscible with water which indicated an absence of typical terpenoid compounds. Finally, a GC–MS analysis of a droplet showed unequivocally the presence of water ( $m/e = 18$ ) and the absence of any terpenoid compounds. However, besides the main peak consisting of water the gas chromatogram displayed an additional rather small peak at longer retention time. It was identified by means of its mass spectral trace showing a molecular ion peak at  $m/e = 118$  and the characteristic fragmentation pattern of succinic acid. The droplets were acidic as derived from their reaction to indicator paper. Obviously, the water droplet inclusions in amber could either originate from splash water and rain, or they could be derived as a reaction product of the chemistry of amber formation.

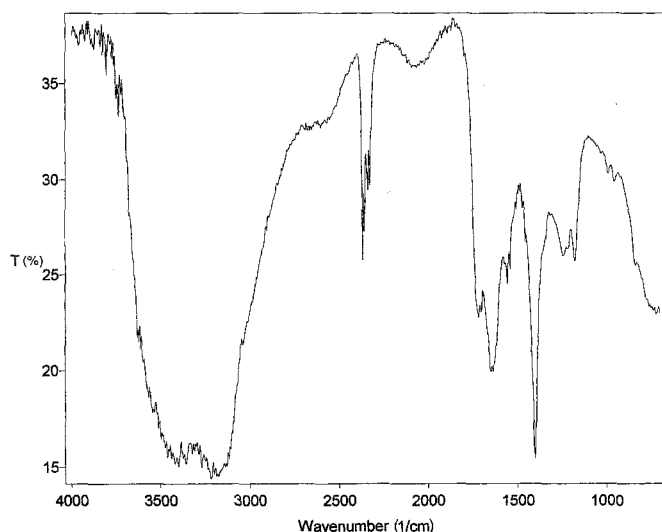


Fig. 1. Micro-FTIR spectrum of an amber inclusion droplet

**Table 1.** Ion contents (ppm; accuracy estimated  $\pm 10\%$  due to the sampling procedure) of the four amber inclusion droplet samples 1–4

	1	2	3	4
NH <sub>4</sub> <sup>+</sup>	717	486	228	1091
Na <sup>+</sup>	499	6	29	9
K <sup>+</sup>	102	20	49	26
Mg <sup>2+</sup>	34	3	25	4
Ca <sup>2+</sup>	49	8	44	30
Cl <sup>-</sup>	302	124	56	<sup>a</sup>
Br <sup>-</sup>	1219	444	301	<sup>a</sup>
SO <sub>4</sub> <sup>2-</sup>	256	8	20	<sup>a</sup>
NO <sub>3</sub> <sup>-</sup>	2563	5	16	<sup>a</sup>
acetate	532	155	24	381
succinate	10890	5140	2460	19990
anion eqvs.	267	101	49	351
cation eqvs.	69	28	19	63
anion/cation	4.5	3.6	2.6	5.6

<sup>a</sup> Content too small to be analyzed quantitatively in presence of the overwhelming amount of succinate

However, in the latter case such droplets should not be exceptional but should occur more or less evenly distributed in ambers. To solve this problem, the contents dissolved in the droplets were investigated to provide evidence for either of these possibilities.

Table 1 contains the analytical results for four exemplaric inclusion droplets (1–4) obtained from four different amber pieces. From the comparison of the qualitative analyses of these four samples it followed immediately that they were quite similar. However, for typical splash water contents *e.g.* Na<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup> from marine, brackish, or lacustrine origin or even rain, which are all present in all the samples, two ions were atypical: the ammonium and the succinate ions.

With respect to the quantitative aspect, the four samples 1–4 differed considerably from each other. Nevertheless, the main features as illustrated in Fig. 2 remained the same. First of all, all four samples consisted mainly of succinic acid dissolved in splash or rain water, which was also corroborated by the proportions of anion to cation equivalents shown in Table 1. Second, bromide ions were present in significant amounts. However, compared to the bromide contents the sodium and chloride levels were found to be rather low. Third, significant amounts of ammonium and acetate ions were also found in all cases.

Thus, we were left to hypothesize about the origin of the water and the various components dissolved in these droplet inclusions. First of all, from the presence of inorganic components dissolved in the water and from the rare occurrence of these droplets in amber one could conclude that the source of water was mostly the inclusion of accidental water droplets from rain or splashes. Accordingly, it could not be the product of chemical processes involved in amber formation.

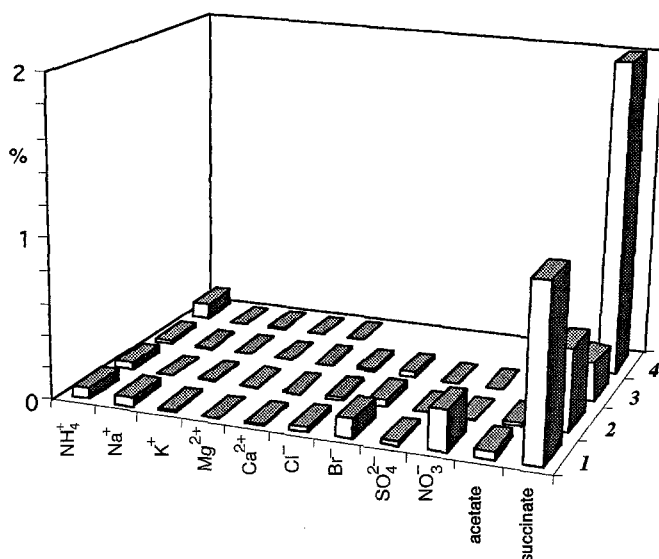


Fig. 2. 3D plot of the contents of samples 1-4

Judged from the main inorganic contents of the droplets, and especially from the significant occurrence of bromide ions, saline splash water and not rain was favored as the source of the droplets. The enrichment of bromide with respect to chloride could be envisaged to be due to multiple addition-elimination sequences of these ions involving terpenoid double bonds of the amber material surrounding the droplet. The presence of ammonium and acetate ions can be explained either by their formation from proteinous materials contained in muddy splash waters or from proteinous materials originating from the sap of the amber producing trees.

The high succinic acid content in the Baltic amber inclusion droplets came as a surprise since it has been amply debated and speculated that succinic acid or its anhydride detected sometimes in ambers could be mainly artifacts from oxidation processes brought about by chemical workup procedures [3, 4]. However, from our results on the inclusion droplets, succinic acid or its anhydride have to be judged as a genuine constituent of amber which, in the course of time, had been extracted into the splash water inclusion droplets. Formic acid, which also could be thought of as a reaction product of amber formation [4, 9], however, could not be detected at all.

## Experimental

The Baltic amber samples (pieces of about 1g of the succinit type) used in this study were obtained from the now shut down *Tagbau Grube Goitsche* in the region of Bitterfeld/Sachsen-Anhalt, Germany. They were of Eocene age and redeposited in the lower Miocene (Aquitain) [1]. The amber pieces were cut in a way to obtain a plane located near to an inclusion droplet. After grinding this plane to minimize the distance to the inclusion droplet, the inclusion was punctured and the contents sampled with a microsyringe. Droplet sizes of the four representative samples 1-4 were 1.0, 5.0, 1.5, and 2.5  $\mu\text{l}$ .

The samples were diluted 50-, 10-, 30-, and 20-fold with doubly distilled water and analyzed for ions by means of capillary electrophoresis using a Beckman P/ACE 2050 instrument with a fused silica capillary of 75  $\mu\text{m}$  inner diameter and 60 cm effective length. The carrier electrolyte for cation separation consisted of 5 mM imidazole + 6.5 mM  $\alpha$ -hydroxyisobutyric acid + 2.5 mM 18-crown-6

at pH 4.5. The separation voltage was 20 kV, and the peaks were detected indirectly at 214 nm. For the anion separations, a mixture of 5 mM sodium chromate + 0.5 mM tetradecyltrimethylammonium bromide + 5% ethylene glycol at pH 9 was used as the electrolyte. The separation voltage was -20 kV, and the peaks were detected indirectly at 254 nm. Alternatively, to allow for an unequivocal discrimination between formate and succinate ions, an electrolyte system consisting of 50 mM NaH<sub>2</sub>PO<sub>4</sub> + 0.5 mM tetradecylammonium bromide at pH 5.6 and detection at 185 nm were employed. The quantitative analytical values (Table 1) were estimated to have a precision of ±10%. A further droplet was directly analyzed on a KBr plate by means of an ATI-Mattson Genesis series FTIR instrument equipped with a Quantum infrared microscope. GC-MS was performed for such a sample by means of an HP 5890 Series 2 gas chromatograph equipped with a HP-5 column (25 m length, 250 µm inner diameter, 0.25 µm film thickness) coupled to a MS-Engine HP 5889 A mass spectrometer.

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