



Vibrational spectroscopy with chromatographic methods in molecular analyses of Moravian amber samples (Czech Republic)



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ABSTRACT

New information on the molecular composition of valchovite (from Boskovice Furrow, Czech Republic) and studlovite (Študlov, near Valašské Klobouky, South-east Moravia, Czech Republic) were obtained using gas chromatography, attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR), and Fourier transformed Raman spectroscopy. ATR-FTIR was coupled to principal component analysis and used for the classification of ambers according to their composition. The archaeological amber originating from a Lusatian Urnfield settlement of the Hallstatt period (Kralice na Hané, near Prostějov, Czech Republic), together with Baltic amber (Denmark), were used to demonstrate that two principal components suffice to classify the samples into distinct groups. Valchovite (Upper Cretaceous) and studlovite (Eocene) did not show compounds and features typical for Baltic amber, but only nonspecific or non-identifiable compounds that are remnants of the original terpenoids. However, although the results of the analytical records did not indicate a more specific plant source, studlovite was classified as belonging to amber classification Class 2, and valchovite, to Class 1b. The results confirmed the higher degree of maturation of studlovite in comparison with older valchovite and the close spectral resemblance between ambers of different ages emphasises the importance of alterations and post-depositional conditions on the character of the resulting amber. Succinic acid, determined as its dimethyl ester, and the 'Baltic shoulder', the well-known markers in ambers of Baltic origin, as well as softening point, or the ratio I1645/I1450 in the FT-Raman spectra, showed that the archaeological samples were derived from Baltic amber, confirming the view that the territory of Moravia was a trading or market center for this material on its way to southern and southeastern Europe.

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1. Introduction

The term amber refers mainly to brittle and hard fossil resins with a high content of succinic acid, originating usually in the Upper Eocene deposits as outcrops on the Baltic Sea shore. However, amber is generally synonymous with all fossil resins that differ geographically in age and chemical composition [1]. Amber has been documented from the late Carboniferous to late Pleistocene sediments throughout the world, predominantly derived from gymnosperm trees.

Rare amber deposits occur in Bohemia and Moravia of the Czech Republic. They are mainly of Cretaceous (Cenomanian) and Tertiary (Eocene, Oligocene, and Miocene) ages, and some of them are known by their mineralogical names duxite, valchovite, neudorfite, muckite [2–3]. In Moravia, the most substantial deposit is of the Eocene amber studlovite, from the Carpathian Flysch Belt (the village of Študlov, near

Valašské Klobouky, South-east Moravia). Amber of such quality and variety of colours suitable for grinding, has not been found anywhere else in the Czech Republic. Additionally, fossil insects (wasps, spiders, ants) were discovered in some pieces of this amber. The basic description was reported in [2] and [4]. The other amber found in Moravia is valchovite (valchovite, walchowite; from Valchov east of Boskovice, central Moravia) of the Upper Cretaceous age, basic investigation about which was reported in [5] and [3]. Baltic amber has also been found in Moravia, although rarely, in glacial deposits of Pleistocene continental ice sheets in the surroundings of Vidnava (Czech Silesia).

Different analytical techniques have been applied to the characterization of amber. Infrared spectroscopy has been most often employed because it was the first technique capable of readily identifying Baltic amber through the presence of a succinic acid peak (so called 'Baltic shoulder') in the infrared spectrum, and was used to simply identify fakes in jewellery. Today, attenuated total reflection (ATR), a sampling technique used in conjunction with infrared spectroscopy with no

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sample preparation and requiring small samples is mainly used for the discrimination of various types of amber [6]. Raman spectroscopy is also a useful and non-destructive method to differentiate between amber specimens of various geological, geographical, and archaeological origins [7–9]. However, although these spectroscopic techniques have advantages, the spectral data may not always give sufficient information for chemical classification of the amber and discrimination of non-Baltic ambers. This is because where discrimination relies only on the presence of the ‘Baltic shoulder’ there are other varieties of European ambers such as gedanite [10], romanite [11], goitschite [12], and American ambers [13,14] that also containing traces or larger amounts of succinic acid. Gas chromatography/mass spectrometry (GC/MS) or pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) are other methods capable of separating a broad range of functional groups or compounds that comprise amber, and the Py-GC/MS method was primarily used to differentiate and classify fossil resins [15]. In fact, the combination of more analytical techniques is a valuable tool in studying amber-related topics in an attempt to better understand amber chemistry, origins, and fossilization processes.

The aim of this study was to provide detailed information on the molecular composition of studlovite and valchovite using GC/MS of resins extracts, Py-GC/MS using *in situ* sample derivatisation with tetramethylammonium hydroxide (TMAH-Py-GC/MS), ATR-FTIR analysis, and FT-Raman spectroscopy analysis. For the preliminary stage of the study, elemental analysis was carried out in order to confirm the type and authenticity of the samples.

The next aim of this study was to find typical representative features of studlovite and valchovite in order to permit correct identification of geological and archaeological amber finds. Reflections on utilisation of the local Moravian fossil resins from Valchov and Študlov for jewellery in prehistoric times can be found in archaeological and mineralogical papers [16–17]. Small decorations e.g. from the Študlov raw material were prepared in recent times. For this reason, typical representative features were sought after to provide evidence that the excavated amber jewellery dated to the Hallstatt Age from Kralice na Hané not only for Baltic amber but also for both Moravian sources. Baltic amber from Burg on Fehmarn was chosen as a reference amber sample for the comparative analyses.

2. Materials and methods

2.1. Samples

Nine samples were analysed: two samples of studlovite (I, II) from the village Študlov (near Valašské Klobouky, South-east Moravia), two valchovites (I, II) from Boskovice Furrow (Czech Republic), two Baltic ambers (I, II) from Burg on Fehmarn (Germany), and three archaeological samples (A, B, C) of unknown provenience from Kralice na Hané (near Prostějov, central Moravia).

The samples of studlovite were found in Eocene deep-sea sediments. The amber-carrying position was formed by dark grey to black sandy claystone or clay sandstone. The analysed amber samples were brown in colour, and were partly transparent. Valchovite accumulated in Cenomanian dark grey claystones of fluvio-lacustrine origin. It formed waxy butterscotch, with opaque grains. Two pieces of brown-yellow, partly transparent amber of known Baltic origin were analysed for comparison of the samples.

2.2. Archaeological background

The territory of Moravia is rich in archaeological finds of amber because it was crossed by the Amber Road, connecting sources on the Baltic Sea shore with lands along the Danube and with Italy. The earliest known archaeological finds were derived from the end of Palaeolithic, connected with the Magdalenian settlement in caves of the Moravian Karst. Amber decorations from the Neolithic in Moravia are not known, which is curious. For the first time relatively abundant amber

artefacts have been found in graves of the Bell Beaker culture (Late Eneolithic). Mass occurrences effectively appeared in the Bronze Age, and especially in the Hallstatt and La Tène periods.

One of the most important archaeological findings is represented by a Lusatian Urnfield settlement from the Hallstatt period, at Kralice na Hané near Prostějov, central Moravia [18]. Two settlement pits were extremely rich in amber – a few thousands small pieces with a total weight of 202.64 g, including finished beds, their rough-outs, waste, small chips and raw material as well. The archaeological features were evidence of local production of amber beds and were unique in the Hallstatt period of the Lusatian Urnfield culture in Moravia. Small amber beds had diameters from 3.6 to 13.5 mm and they were 1.5 to 9 mm high. The most prevalent were symmetrical or asymmetrical two-conical beds. The large amount of production waste and chips allowed us to apply destructive analyses for the determination of amber provenience.

2.3. Methods

The elemental organic composition was determined using a CHNS/O microanalyser Flash FA 1112 (ThermoFinnigan). Softening point was determined using the Vicat method (Laget).

Powdered samples (<0.2 mm) were dissolved in dichloromethane (DCM) and analyses were performed using a Trace GC Ultra-DSQ II (ThermoElectron) instrument equipped with a TR-5MS column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 36 °C (3 min) to 120 °C (12 °C/min), to 250 °C (6 °C/min), to 300 °C (3 min) (12 °C/min). Samples were injected in splitless mode, the injector temperature was set at 200 °C and helium was the carrier gas. Mass spectra were obtained by scanning from *m/z* 45 to 500 in full scan mode. α(H)-androstane in isooctane was used as an internal calibration standard. TMAH-Py-GC/MS analyses were performed using a Pyroprobe 5150 (CDS Analytical) pyrolysis unit connected to the GC/MS instrument described above. Samples of powdered amber, together with 10 μL of a 25% aqueous solution of TMAH for methylation trials were heated at 610 °C for 20 s. The TR-5MS column was used and the temperature programme of the columns was set from 40 °C (3 min) to 210 °C at 8 °C/min, and continued to 300 °C (2 min) at 20 °C/min. For data processing, the Xcalibur software (ThermoElectron) was used. Components were assigned from retention times and comparison with mass spectra from the National Institute of Standards and Technology spectral library.

Fourier transformed infrared (FTIR) spectra of the bulk samples were collected on a Nicolet 6700 FTIR spectrometer (Thermo Nicolet Instruments Co.) with a N₂ purging system. Spectra were acquired using a single reflection ATR GladiATR accessory equipped with a single bounce diamond crystal (angle of incidence 45°). Each sample spectrum averaged 64 scans and the resolution was 2 cm⁻¹. The spectra were plotted against a single-beam spectrum of the clean ATR crystal and converted into absorbance units by ATR correction. Data were collected over a wavenumber range 4000–400 cm⁻¹.

FT-Raman spectra were obtained using a Nicolet 6700 instrument with the NXR 9650 FT-Raman module attached and Nd³⁺/YVO₄ near-infrared excitation at 1064 nm. Spectra were recorded at 4 cm⁻¹ resolution from 1024 accumulated scans. A nominal laser power of about 0.1 W was used with a spot size of 50 μm.

The discriminant analysis was carried out using TQ Analyst software ver 9.3.107 (Thermo Fisher Scientific Inc.). The infrared spectroscopic data for discriminant analysis were fitted by mean-centering. Two-point baseline (1870, 1505 cm⁻¹) was used for the wavenumber region of 1870–1505 cm⁻¹. A constant pathlength was used for calculations. The Mahalanobis distance was used to formulate the distance between clusters. The number of principal components used for calculation was 10.

3. Results and discussion

The composition of fossil resins can vary depending on their botanical source, and usually is: carbon, 67–87%; hydrogen, 8.5–11%; oxygen,

15%; sulphur, 0–0.5%. All the samples tested fell within this range (Table 1).

3.1. GC/MS analysis

The total extracts of ambers contained mainly mono-, sesqui- and diterpenoids (Fig. 1, Table 2).

Straight-chain alkanes and naphthalenes were present in the studlovite extracts. The only compound that preserved the terpenoid skeleton of its original source was a sesquiterpene of cadinene structure (204). There were no similarities with chromatograms of other sample extracts.

In the valchovite extracts, cyclic diterpenes, dihydro-*ar*-curcumene and tetrahydronaphthalenes were identified and correspond with the previous result [5]. Tetrahydronaphthalenes are degraded diagenetic products of various terpenoids, and dihydro-*ar*-curcumene is suggested to be derived from bisabolane sesquiterpenoids, derived primarily from higher plants [19]. The predominant compounds (16–19) (Table 2) were interpreted as diterpenes according to their molecular weight and patterns of mass spectrometric fragmentation [20].

Monoterpenoid compounds in the samples of Baltic amber included camphene, fenchone, fenchol, camphor and borneol. The camphor peak was the most abundant of the monoterpenes. This composition corresponds with known results [21–23,11].

The archaeological samples of unknown provenience from Kralice na Hané revealed very similar signals to the Baltic amber results, with the exception that camphor was not the most abundant in all extracts, and borneol was predominant in two cases. The monoterpenoid borneol occurs in modern conifers and can be preserved unchanged in the amber, as described from several other ambers of the Cretaceous and Miocene ages [24–25]. Camphor is an oxidized form of borneol.

3.2. TMAH-Py-GC/MS analysis

Studlovite revealed a similar fingerprint as valchovite in the TMAH-Py-GC/MS spectra: cyclic compounds were identified in the pyrograms. The general fingerprint of studlovite and valchovite was different from Baltic amber. The compounds identified in the Baltic amber extracts (fenchol, camphor, borneol), were also detected in the pyrolysates of Baltic amber, and also of the archaeological ambers (Fig. 2).

The pyrolysates showed an abundance of cyclic products derived from mono-, sesqui- and diterpenoid precursors. The dominant constituent of Baltic ambers was succinic acid (in the spectra as its dimethyl ester). These results are in agreement with published data [22,26].

3.3. ATR-FTIR analysis

All ATR-FTIR spectra were dominated by bands belonging to the antisymmetric and symmetric stretching vibrations of the aliphatic C—H bonds of methyl, methylene, and methine groups in the wavenumber region 3000–2700 cm^{-1} (not shown here). The bands of bending vibrations of CH_2 and CH_3 groups can be found at 1454 and 1377 cm^{-1} (Fig. 3a). Bands at 3078, 1646, and 887 cm^{-1} , which are attributed to

C—H stretching, C=C stretching, and $=\text{CH}_2$ out-of-plane bending vibrations in exocyclic methylene groups, were detectable in the spectrum of Baltic amber and in all spectra of the archaeological ambers from Kralice na Hané (Fig. 3b). The intensities of exocyclic bands are dependent on the maturity and degree of oxidation of the ambers [5,27]. In the spectrum of valchovite, a low intensity band of exocyclic methylene groups can be found at 889 cm^{-1} .

There are relatively large differences in the region of the stretching vibration of carbonyl groups of the individual amber samples. Using the second derivative and curve-fitting of the spectra (Fig. 4), the positions of ketone and carboxyl bands were found at 1722 and 1703 cm^{-1} for studlovite, while for valchovite the values were 1732 and 1703 cm^{-1} . In the case of Baltic amber, the ester and carboxyl bands at 1739 and 1709 cm^{-1} were found. The bands between 1780 and 1770 cm^{-1} can be ascribed to anhydrides and the bands around $\sim 1600 \text{ cm}^{-1}$ may be assigned to vibrations of C=C bond in aromatic rings.

The fingerprint zone shows many differences among analysed samples. The ATR spectrum of the studlovite shows bands at 1239, 1185, 1139, 1072, 1029, 979, 869 and 829 cm^{-1} . In the spectrum of the valchovite, bands at 1240, 1160, 1027, 981, 889 and 855 can be found. The Baltic amber and archaeological sample spectra contain the typical 'Baltic shoulder', the horizontal flat feature between 1250 and 1175 cm^{-1} , and an associated band at 1161 cm^{-1} [27–28]. The 'Baltic shoulder' has been assigned to the absorption of succinic acid ester bonds of polyester-like structures [29]. This horizontal region can exhibit a spectral slope of oxidized amber samples [27,30].

3.4. Discriminant analysis

Principle component analysis (PCA), one of the most common multivariate methods used in infrared spectroscopy, was applied to quantify the probability with which ambers can be assigned to individual classes. PCA reduces multivariate data by transformation into orthogonal components, which are linear combinations of the original variables. Synthetic variables, designated as principal components, are set so as to describe the maximum variation in the data set.

The first three principal components (PC1, PC2, PC3) described the most important structural information on the samples: PC1 described 48.85%, PC2 26.57%, and PC3 13.29% of the spectral variance.

Discriminant analysis using Mahalanobis distance was applied on PCA scores as a very useful way of determining the similarity or dissimilarity of a set of samples and an unknown sample. The closer each Mahalanobis distance value is to zero, the better the match [31]. Results of the amber analysis were presented as distance plots in Mahalanobis distance units in Fig. 5: the x-axis shows the distance to the class of valchovites while the y-axis shows the distance to the class of Baltic ambers. The results revealed that analysed samples can be divided into three distinguishable clusters. The first cluster is represented by the valchovite samples and the second by studlovite samples. The third cluster contains a class of Baltic ambers and archaeological samples. It can be therefore concluded that the archaeological samples were not made of valchovite or studlovite.

3.5. FT-Raman spectroscopy analysis

In the FT-Raman spectra of ambers (Fig. 6), the ratio of the intensities of bands at 1645 cm^{-1} (C=C stretching of double bonds), and 1450 cm^{-1} (aliphatic C—H bending), was suggested to indicate the maturity and degree of oxidation of the fossil resin samples [3,32–33]. This ratio decreases with increasing maturation due to the loss of double bonds. The ratio I_{1645}/I_{1450} , presented in Table 4, together with band positions and their vibrational mode assignments [3,8,32], vary, in the case of the archaeological samples, between 0.63 and 0.76. These values were similar to the ratios of Baltic ambers that lie between 0.65 and 0.78. In the case of valchovite, the ratios were between 0.46 and 0.53

Table 1

The elemental composition (wt.%) of samples (dry basis).

Sample	Abbreviation	Locality	C	H	N	S
Studlovite I	STU I	Študlov	69.7	8.1	0.0	0.1
Studlovite II	STU II	Študlov	69.5	8.0	0.0	0.0
Valchovite I	VAL I	Boskovice Furrow	71.6	8.9	0.1	0.0
Valchovite II	VAL II	Boskovice Furrow	73.0	9.5	0.0	0.0
Baltic amber I	BALT I	Burg on Fehmarn	69.4	8.8	0.2	0.0
Baltic amber II	BALT II	Burg on Fehmarn	79.1	9.2	0.2	0.0
Archaeological sample A	ARCH A	Kralice na Hané	72.2	8.6	0.2	0.0
Archaeological sample B	ARCH B	Kralice na Hané	71.3	8.8	0.1	0.0
Archaeological sample C	ARCH C	Kralice na Hané	72.0	8.9	0.1	0.0

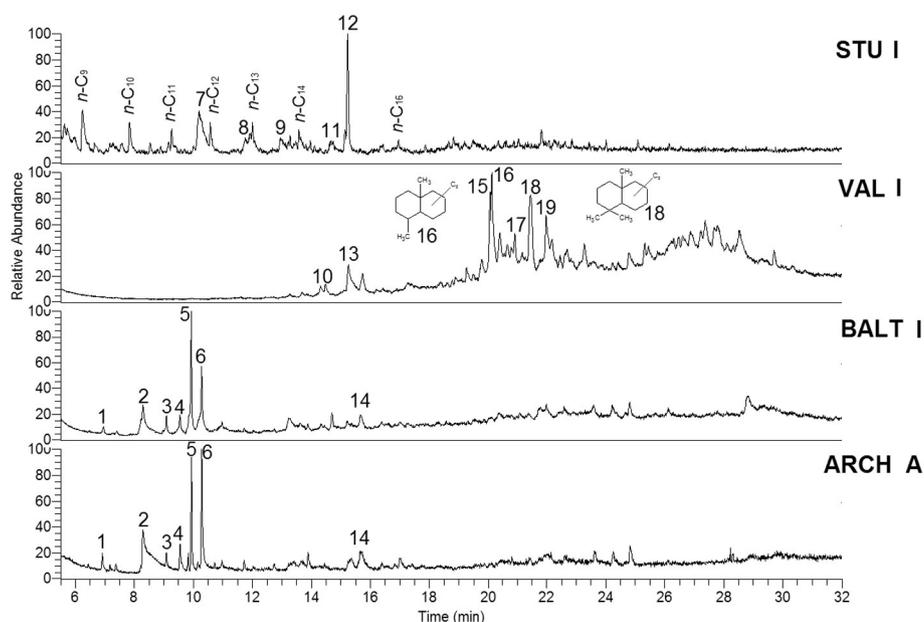


Fig. 1. Representative TIC chromatograms of total sample extracts. The numbers refer to Table 2, and *n*-alkanes are assigned.

and corresponded to the published value of 0.57 [3]. The lowest value of 0.30 was for studlovite, which had the highest degree of maturation (oxidation).

In the spectral region between 800 and 600 cm^{-1} , important differences in the band positions and intensities were found: characteristic Raman spectra of the archaeological and reference Baltic ambers displayed four bands at about ~ 740 , ~ 720 , ~ 695 and ~ 650 cm^{-1} , which can be assigned to stretching vibrations of C—C and C—S bonds. The Raman spectrum of studlovite in this wavenumber region showed a doublet at 740 and 722 cm^{-1} while the valchovite spectrum exhibited only one band at 718 cm^{-1} .

3.6. Characteristic features of studlovite, valchovite, and Baltic amber

The chemical composition of ambers consists of a complex mixture that mainly includes terpenoids. This specific composition depends on several factors such as paleobotanic source, diagenetic alterations, paleoclimate, and geological history.

Valchovite is the amber from the Cenomanian age that was one of the warmest periods of the Phanerozoic, and corresponds to one of the most important transgressive episodes of the geological record. The Cenomanian floras are known for their biodiversity [34], exhibiting high diversification of angiosperms, together with numerous gymnosperms.

Studlovite is younger — of Eocene age, but the pyrogram fingerprint was close to the Cenomanian sample, and could suggest a similar plant source. In the Paleogene, northern European land was characterized by stable subtropical-tropical climates, when warm and humid conditions could increase resin exudation, as is known from recent forests in a warm subtropical or tropical climate. The Eocene era includes the warmest climate in the Cenozoic Era and the warming might have caused an enhanced outflow of resin.

Only cycloalkanes were found in pyrograms of studlovite and valchovite; other peaks were not possible to identify. The cycloalkanes are analytical products from terpenoid structures that formed the macromolecular amber matrix. The high temperature during pyrolysis caused a cracking reaction and cycloalkanes were produced because

Table 2

Identified compounds and their concentrations in the sample extracts (mg/g of solid sample).

Compound	STU		VAL		BALT		ARCH		
	I	II	I	II	I	II	A	B	C
Camphene (2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane)					0.02	0.11	0.05	0.03	0.13
o-Cymene (1-methyl-4-(1-methylethyl)benzene)						0.31		0.18	
Fenchone (1,3,3-trimethylbicyclo[2.2.1]heptan-2-one)					0.03	0.05	0.03	0.02	0.21
Fenchol (1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol)					0.04	0.06	0.05	0.04	0.42
Camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one)					0.27	0.31	0.28	0.16	0.71
Borneol (1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol)					0.16	0.19	0.23	0.21	1.53
Naphthalene	0.14	0.12							
Methylnaphthalene	0.06	0.04							
Biphenyl	0.05	0.03							
Dihydro- <i>ar</i> -curcumene (1-(1,5-dimethylhexyl)-4-methyl-benzene)			0.04	0.03					0.8
Methylbiphenyl	0.04	0.02							
Sesquiterpene (204)	0.12	0.10							
Trimethyltetralin (1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene)			0.1	0.16					
Cadinatriene (8-isopropyl-2,5-dimethyl-1,2,3,4-tetrahydronaphthalene)					0.02	0.81	0.12	0.45	1.08
5,6,7,8-tetramethyl-1,2,3,4-tetrahydronaphthalene			0.23	0.21					
C18-bicyclic diterpene (248)			0.79	0.77					
C18-bicyclic diterpene (248)			0.05	0.08					
C19-tricyclic terpene (262)			0.53	0.5					
C18-bicyclic diterpene (248)			0.25	0.34					

Table 3
Compounds identified in pyrograms of fossil resins and their relative abundances.

Compound	Studlovite		Valchovite		Baltic amber		Archaeological samples		
	I	II	I	II	I	II	A	B	C
Dimethylcyclopentene	6.6	5.9	7.4	6.9					
Dimethylcyclohexene	16.9	17.7	19.4	20.8	5	9.3	5	5.3	3.2
Dimethylcyclohexadiene	5.3	5.9	2.2	3.1					
Dimethylcyclohexadiene	4.9	3.8	7.1	5.5					
Tetramethylcyclopentene	8.4	8.1	8	6.1	2.3	6.6	3.2	3	2.1
Succinic acid dimethyl ester					60.9	40.3	69.1	68.2	61.4
Dimethylbenzene	6.1	6	5.9	6.5					
Tetramethylcyclohexene	5.7	5.6	10.9	10.3	3.6	6.1	3.9	4.3	1.6
Trimethylbenzene			10.4	3					
Fenchol					2.5	1.6	1.4	1.2	3.8
Camphor					2.5	1.3	2.8	2.9	4.9
Borneol					2.4	6	2.9	4.3	5.1
Tetramethylcyclopentene	4.1	6.1	2.1	4.5					

the macromolecular matrix was formed by substituted (hydrogenated) molecular units.

The cycloalkanes are nonspecific compounds, but examining the extract analysis of studlovite, next to naphthalene, the other more intensive compound was a sesquiterpene (204) that had molecular spectra close to a cadinene structure. Together with the absence of succinic acid (dimethyl succinate) in the pyrolysate, it can be suggested that studlovite belongs to amber classification Class 2 [35]. The natural precursors of studlovite were suggested to be deciduous trees [4], however this assumption was made on the basis of a missing succinic acid peak in the FTIR spectra. This is insufficient proof for this conclusion. The absence of plant triterpenoids (such as oleananes) in studlovite extracts is more believable as evidence of a conifer as the source of amber.

Diterpenes (16–19) (Table 2) were predominant in valchovite extracts and the presumed precursor was the normal labdatriene (bicyclic) derived communic acid. Succinic acid (dimethyl succinate) in the pyrolysate was absent, and this suggests that the valchovite belongs to amber classification Class 1b [35], based on polymers and copolymers of labdanoid diterpenes. Valchovite was assumed to originate from the Araucariaceae family of coniferous trees (*Dammarophyllum*) [3].

The identified and characteristic features of ambers that can be used for their identification are summarised in Table 5.

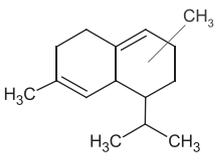
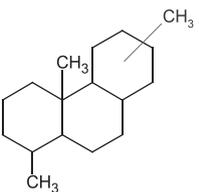
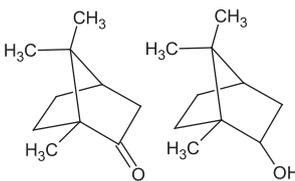
Differing results of extract analysis and pyrolysis are not unusual. Amber is formed as a result of fossilization of plant resin and involves oxidation and polymerization of original oxygenated hydrocarbons. The more the components are polymerized, the closer the resin comes to the features of true amber in hardness and solvent insolubility. The

Table 4
Assignment of vibrational modes for the FT-Raman spectra of the studied ambers.

Studlovite		Valchovite		Baltic amber		Archaeological samples			Assignments
I	II	I	II	I	II	A	B	C	
2927	2928	2931sh	2931	2932	2932	2932	2931	2931	$\nu(\text{CH}_2, \text{CH}_3)$
2869	2870	2872	2874	2874	2870sh	2873	2872	2872sh	$\nu(\text{CH}_2, \text{CH}_3)$
2846	2846	2848				2850sh	2850sh		$\nu(\text{CH}_2, \text{CH}_3)$
1670sh	1671sh	1712sh		1721sh	1717sh	1721sh	1726sh	1721sh	$\nu(\text{C}=\text{O})$
1650	1650	1647	1651	1645	1644	1644	1645	1647	$\nu(\text{C}=\text{C})$
1609	1611	1611sh		1607sh	1609sh	1611	1609	1611	$\nu(\text{C}=\text{C})_{\text{ar}}$
1475sh	1475sh								$\delta(\text{CH}_2, \text{CH}_3)$
1443	1445	1450	1447	1450	1447	1450	1450	1446	$\delta(\text{CH}_2, \text{CH}_3)$
1378	1380								$\delta(\text{CH}_2, \text{CH}_3)$
1356	1355	1356	1355	1357	1357	1357	1356	1356	$\delta(\text{CH}_2, \text{CH}_3)$
1298	1297	1296	1300	1299	1297	1297	1298	1299	$\delta(\text{CH}_2, \text{CH}_3)$
				1245	1247	1245	1245		$\delta(\text{CH}_2, \text{CH}_3)$
1202	1203	1204	1206	1202	1204	1203	1204	1202	$\delta(\text{CCH}_2, \text{C}-\text{O})$
1139	1142	1144		1145	1141				$\nu(\text{C}-\text{C})$
1108	1109			1109	1102	1107	1108		$\nu(\text{C}-\text{C})$
		1094	1095						$\nu(\text{C}-\text{C})$
1061	1063	1064		1064	1064	1062	1063	1065	$\nu(\text{C}-\text{C})$
		1030	1035	1032	1030	1034	1033		$\nu(\text{C}-\text{O})$
		1001				1001	1001		$\nu(\text{C}-\text{C})$
977	976	976	976	976	976	978	976	973	$\rho(\text{CH}_2, \text{CH}_3)$
		939		939	938	936	940	934	$\rho(\text{CH}_2)$
885	883	878	879	881	882	882	880	883	$\nu(\text{COC})$
833	830			829	833	826	827	800	$\nu(\text{COC}, \text{C}-\text{C})$
740	742			743	740	745	746	749	$\nu(\text{CC})$ isolated
722	720	717	718	720	719	715	721	714	$\nu(\text{CC})$ isolated
				694	694	694	695	695	$\nu(\text{CC})$ isolated
			657	650	651	650	650	644	$\nu(\text{CC})$
				588		588		609	$\nu(\text{C}-\text{S})$
556	556	554	559	557	556		555	557	$\nu(\text{S}-\text{S})$
		515	502	503	505	503	503	500	$\nu(\text{COC}, \text{S}-\text{S})$
449	449		454	451	452	451	454	453	$\delta(\text{CCO})$
384	380	359		363	362		373	338	$\delta(\text{CCO})$
310	310	307		258	303		310	305	$\delta(\text{CCO})$

sh – shoulder.

Table 5
Features of the studied studlovite, valchovite, and Baltic amber.

	Studlovite	Valchovite	Baltic amber
Age	Paleogene/Eocene (56 to 33.9 Ma)	Cretaceous/Cenomanian (93.9–100.5 Ma)	Paleogene/Eocene (56 to 33.9 Ma)
Colour	Brown, partly transparent	Butterscotch, opaque	Brown, partly transparent
Softening point*	180°C	165°C	155°C
Elemental analysis* (H/C, O/C)	1.40, 0.24	1.53, 0.19	1.46, 0.17
GC/MS (More intensive compounds)	Sesquiterpene (204) 	C18-diterpane (248) 	Camphor, borneol 
TMAH-Py-GC/MS (typical compounds)	Cycloalkanes	Cycloalkanes	Succinic acid dimethyl ester
FT-Raman spectroscopy* (ratio I_{1645}/I_{1450}) ^a	0.3	0.48	0.72
Class (Andersson et al., 1992)	Class 2	Class 1b	Class 1a

*average value

^a– ratio I_{1645}/I_{1450} baseline regions points for intensity measurement: 1645 (1825, 1510), 1450 (1510, 1400) cm^{-1}

TMAH-Py-GC/MS method breaks the amber polymer into simple smaller molecular components providing useful structural information, however, these only represent a portion of the original macromolecular unit. Non-polymerising and occluded compounds in ambers are also present. They are not bound, just entrapped in the macromolecular network of the ambers, and identifiable by GC/MS of sample extracts.

Resins do not have a sharp melting point but a softening point, the temperature at which a material softens sufficiently to allow significant

flow under small stress. The ambers differ only little, however for studlovite this point was a little higher than others. This corresponds to the more hydrogenated structure of this amber and its complex structure. Elemental analysis did not reveal useful differences among the studied samples, and only the lower value for H/C ratio of studlovite corresponded with the substituted structure of this amber.

The typical compounds (fenchol, camphor, borneol) were identified in the extracts and pyrolysates of Baltic amber (Fig. 2). According to the

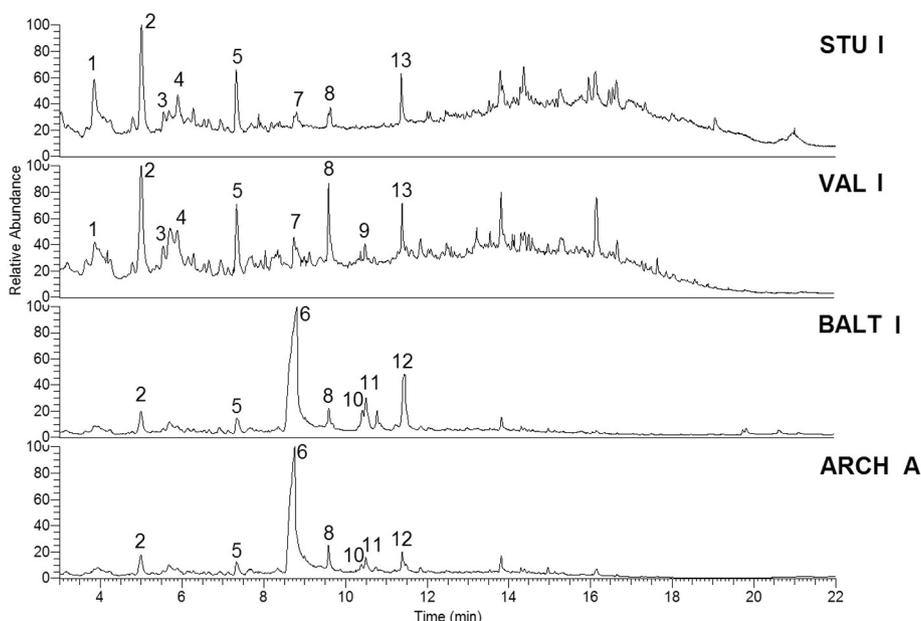


Fig. 2. Representative TMAH-pyrolysis-GC/MS total pyrograms of the ambers. The peak assignments correspond to the product list in Table 3.

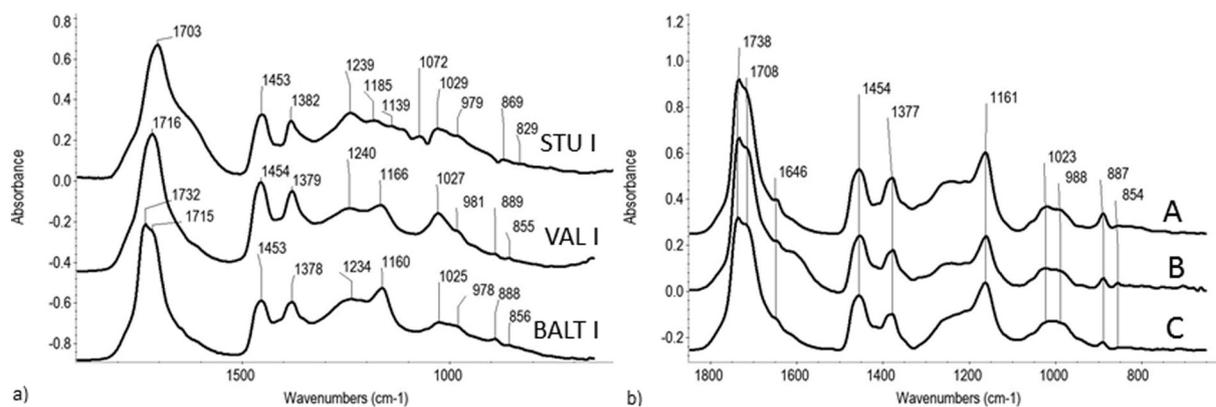


Fig. 3. Representative ATR-FTIR spectra of a) studlovite, valchovite and Baltic amber, and spectra of b) the archaeological ambers (A, B, C).

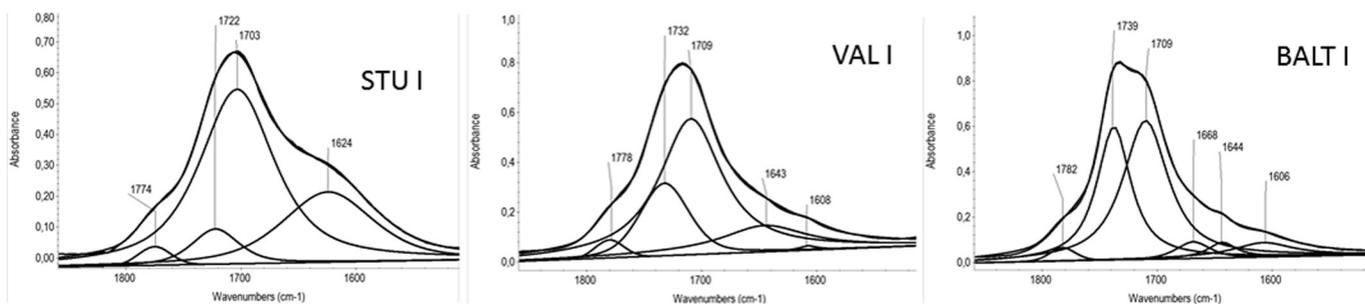


Fig. 4. Curve-fitted carbonyl stretching regions of the studlovite, valchovite and Baltic amber.

classification system of Anderson and co-workers [35], the samples are composed of Class 1a type, which include Baltic amber (and related European ambers), derived from resins based primarily on polymers of communic acid and communol. Baltic amber has never been found in its original stratigraphical position but has been linked to resin production in forests dominated either by the conifer family Araucariaceae (*Agathis*-like), Pinaceae (*Pseudolarix*, *Pinus*) [1, 36] or Sciadopityaceae (*Sciadopitys*-like) [37].

4. Conclusion

Careful analysis of amber materials resulted in the identification of a series of compounds and spectral signals that can distinguish a sample of amber from other types. These compounds and signals included succinic acid, determined as its dimethyl ester, and the 'Baltic shoulder', which are well known markers in ambers of Baltic origin, as well as other features like softening point, or the ratio I_{1645}/I_{1450} in the FT-Raman spectra.

This features, together with resemblance of chromatograms, pyrograms, FT-spectra, and the results of the discriminant analysis of the archaeological samples of unknown provenience from Kralice na Hané, revealed their Baltic origin. Valchovite of the Upper Cretaceous age and studlovite of the Eocene age did not show compounds typical

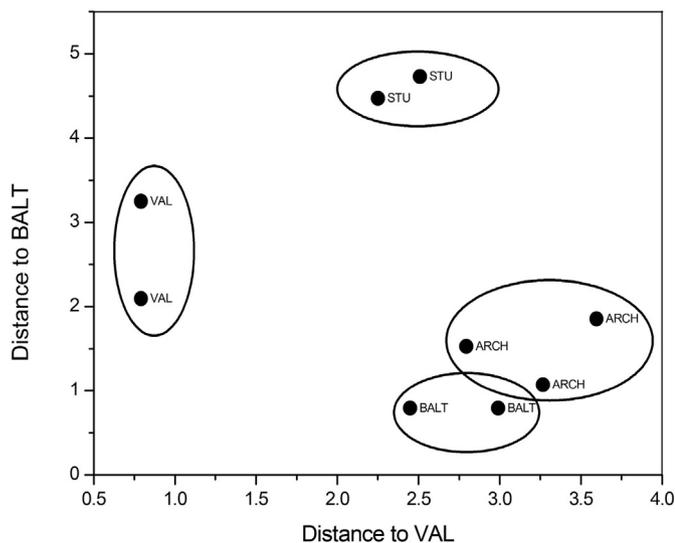


Fig. 5. The Mahalanobis distance plot for four sourced amber samples.

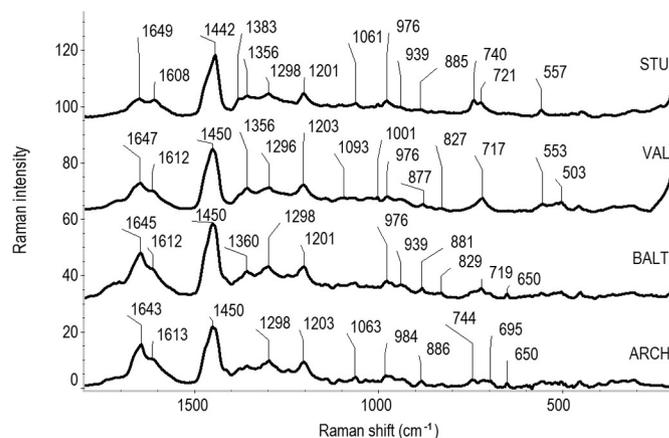


Fig. 6. Representative FT-Raman spectra of ambers in the wavenumber region 1830–600 cm^{-1} .

for Baltic amber and not such characteristic features. Only nonspecific or non-identifiable compounds that are remnants of the original terpenoids were shown in the analytical records and do not indicate a more specific plant source for studlovite and valchovite.

For the first time, the IR and Raman spectra for studlovite, the Eocene amber from the Carpathian Flysch Belt collected in Študlov near Valašské Klobouky in Moravia, have been presented together with GC/MS results. These results confirm the higher degree of maturation of studlovite in comparison with older valchovite (Upper Cretaceous age). The close spectral resemblance between ambers of different ages emphasises the importance of effects of alterations and post-depositional conditions on the character of the resulting amber.

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