SOME POSSIBILITIES
OF THIN LAYER CHROMATOGRAPHIC ANALYSIS
OF THE MOLECULAR PHASE
OF BALTIC AMBER AND OTHER NATURAL RESINS

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SUMMARY

TLC has been used for comparative investigation of the group composition of ethanol extracts obtained from natural resins of different origin and geological age. The main object of the analysis was an extract of Baltic amber from the tertiary period. An extract of an older (cretaceous period) fossil resin from Spain was also analysed, as also were several contemporary resinous substances (Canada balsam, pine resin, and dammar resin).

A variety of visualisation reagents was used both for general detection and for detection of characteristic groups of compounds, for example terpenes, unsaturated compounds, and carboxylic acids. The characteristics of the chromatograms obtained suggest that TLC has potential as an auxiliary tool for classification of natural resins and, especially important, fossil resins.

INTRODUCTION

Baltic amber is called also "succinite", after the Latin word *succus* meaning "sap", because substantial resources of this organic mineral, known mainly from the Baltic sea region, have been formed as a result of the abundant secretion of this resin by trees. Of the miscellaneous fossil resins which occur in various regions of the world Baltic amber is one of the most interesting, because of properties which promote its widespread use. Besides its good attributes as jewellery and its technological usefulness (e.g. in the production of special varnishes), Baltic amber also has properties which result in the bio-stimulation of organisms. It has been stated, for example, that succinic acid isolated from Baltic amber can

stimulate human [1] and plant organisms, and can contribute to an increase in the yield of some cultivated plants [2]. Raw Baltic amber contains 3–8% succinic acid [3]; this is one of main distinguishing properties of this fossil resin. The molecular phase of Baltic amber also contains other compounds, for example terpenoids, with a variety of therapeutic activity, and ethanol extracts of amber have been used therapeutically for a long time. Broader investigation of the soluble components of Baltic amber might, therefore, lead to important applications.

One example of the purpose of chemical analysis of resins might, therefore, be deduction of the relationship between the compounds detected and their role in protecting the source tree. The unsaturated compounds, for example, probably participate in solidification of resins by polymerisation after secretion on a trunk surface.

Another goal of investigation of Baltic amber and other natural resins of different geological age is comparative analysis for characterisation of their similarities and differences. This might help clarify the phytogenesis of many fossil resins. An example of the complexity of this problem is continuing discussion on the phytogenesis of Baltic amber, as described by Beck [4]. Hypotheses about the origin of this amber from coniferous trees of the Pinaceae family, broadly accepted by hundreds of years, have been challenged in recent decades in favour of other coniferous trees of the Araucariaceae family.

The diversity of the resins and their structure and the various objectives of the studies necessitate the use in these investigations of a variety of analytical methods, including chromatographic techniques, although because of the complexity of the macromolecular structure of resins, their complete analysis by use of chromatographic methods is impossible. Extraction must be performed to isolate the molecular components filling the macromolecular network of the resins. This molecular phase characterises the resins indirectly, although with significant relation to their whole chemical structure.

The goal of this work was to use thin-layer chromatography (TLC) for comparative analysis of ethanol extracts of Baltic amber and other natural resins of different genesis and geological age. TLC was chosen on the basis of results obtained in other work [5–9]. These studies clearly indicated the usefulness of TLC for general, mainly comparative, analysis of resins. In the work a variety of mobile phases and visualisation reagents was used for detection of a series of group components in the molecular phase of the resins analysed. A preliminary attempt has been made to

relate group composition similarities and differences to the genesis and diagenesis of the parent resin samples.

EXPERIMENTAL

Characteristics of the Samples Analysed

Baltic amber (succinite), fossil resin from the tertiary period (approximately 40 million years ago) is probably derived from the Pinaceae family of trees [10] (there is no evidence yet of genesis from Araucariaceae [4]). An older fossil resin from Spain (Galicia) was from the cretaceous period (approx. 90–100 millions years ago); its phytogenesis is unknown. Canada balsam is a contemporary resinous secretion of *Abies balsamea*, from the Pinaceae family of trees. *Pinus silvestris*, a source of pine resin, belongs to the same family. The dammar resin used in this work was a secretion from a deciduous tree (*Dammara orientalis*, from the Dipterocarpaceae family), in contrast with the other resins analysed here, which were derived from coniferous trees. Both the fossil resins and the dammar resin were extracted in a Soxhlet apparatus, with ethanol as a solvent. Canada balsam, because of its high solubility, was extracted directly by stirring with ethanol in a glass flask at ambient temperature. Pine resin is completely soluble in ethanol. The extracts were dissolved in dichloromethane (concn 0.15 g cm^{-3}) and applied to chromatographic plates as spots.

Chromatographic Conditions

TLC was performed on 20 cm × 20 cm glass plates precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck); before use plates were activated at 105°C for 30 min. The mobile phase used for analysis of carboxylic acids and α-hydroxy and α-keto acids was dichloromethane–methanol, 10:1 (*v/v*). Hexane–benzene–methanol, 2:6:1 (*v/v*) was used for analysis of other compounds; this mobile phase was adopted on the basis of results from other work [7], but modified somewhat by replacing petroleum ether with hexane.

TLC was used in this work for general and comparative characterisation of the samples and for detection of terpenes, unsaturated compounds, carboxylic acids, α-keto acids, and α-hydroxy acids. Chemical agents for visualisation of chromatograms were prepared in accordance with the Merck catalogue for TLC [11]. The general visualisation reagents phosphoric and sulphuric acids were also used. Fluorescence light excited by a UV-254

nm lamp was also used for additional identification in analysis of unsaturated compounds. R_F values (calculated as averages from three analytical results) and spot colour were used for comparison of the chromatograms obtained.

RESULTS AND DISCUSSION

Terpenes were detected in extracts obtained from Baltic amber and from the contemporary resinous substances pine resin, Canada balsam, and dammar resin by using a solution of anisaldehyde as visualisation reagent. Anisaldehyde is usually used for detection in TLC analysis of terpenes in plant extracts [12]. The reagent was prepared in accordance with literature data [11, p.6] and red spots were expected for detected terpenes. Retention coefficients estimated for spots of different red hues – rose, wine red, and maroon – on the developed chromatograms are presented in Table I.

Table I

Detection of terpenoid compounds by use of anisaldehyde as visualisation reagent. Spot colours and retention coefficients R_F were determined from thin-layer chromatograms obtained from ethanol extracts of Baltic amber, Canada balsam, dammar resin, and pine resin

Sample extract	Retention coefficient (R_F) and spot colour
Baltic amber	0.96, wine red; 0.87–0.62, tailing, dark-rose
Pine resin	0.94, wine red
Canada balsam	0.94, 0.87, and 0.81, maroon; 0.66, rose
Dammar resin	0.93, 0.88, dark-rose; 0.67, rose

Terpene components of Canada balsam extract were observed as spots with intense maroon colours (R_F 0.94, 0.87, and 0.81) and with large areas in relation to those of other spots on the same chromatogram. There was also a smaller, rose, spot at R_F 0.66. On the chromatogram of the extract obtained from dammar resin (derived from a deciduous tree) the spots were less numerous and of lower colour intensity than for the chromatogram obtained from the extract of Canada balsam (from a coniferous tree). There was only one reddish spot, at R_F 0.94, on the chromatogram obtained from the pine resin (Table I). The chromatogram obtained from the extract of Baltic amber was indicative of a greater variety of terpenes; it contained a single spot at R_F 0.96 and a tailing spot from R_F 0.87 to

0.62. Comparison of these results suggests that the chemical composition of terpene compounds in extracts of the older (tertiary) Baltic amber resin is more varied. This trait might be a result of transformation of the primary chemical structure. Various reactions, for example oxygenation, condensation, or polymerisation, could be the reason the structure of fossil resins is more complex than that of contemporary resins.

Unsaturated compounds were detected with fluorescein–bromine reagent, again prepared in accordance with the literature [11, p.41]. Chromatographic data obtained for unsaturated compounds detected in all the samples are listed in Table II.

Table II

Retention coefficients and visual and fluorescence colour (λ_{exc} 254 nm) of spots of unsaturated compounds on TLC chromatograms obtained from ethanol extracts of Baltic amber, Spanish resin, Canada balsam, pine resin, and dammar resin

Sample extract	R_F and colour of spots: visual/fluorescence
Baltic amber	0.97 rose/orange; 0.68 rose/orange; 0.59 yellow/yellow; 0.40 rose/yellow; 0.28 rose/purple; 0.17 yellow/purple; 0.04 yellow/yellow
Spanish resin	0.95 rose/(yellow+purple); 0.67 rose/orange; 0.58 yellow/yellow; 0.39 rose/yellow; 0.29 rose/purple; 0.17 yellow/purple
Canada balsam	0.94 brown/brown; 0.65 yellow/purple
Pine resin	0.61 yellow/(yellow+purple); 0.28 yellow/yellow; 0.09 yellow/yellow
Dammar resin	0.94 yellow/yellow

For extracts of contemporary resins a simple distribution of intense spots from unsaturated compounds was observed. The simplest was that obtained from the extract of dammar resin, derived from a deciduous tree. The unsaturated compounds present in pine resin were the most polar of those present in the contemporary resins. The greater variety of unsaturated compounds in the extracts of the older, fossil resins (Baltic amber and Spanish fossil resin) might be a result of transformation of the primary structure by the various rearrangement, polymerisation, or partial oxygenation processes mentioned above. It is well known, for example, that unsaturated bonds undergo oxygenation processes relatively easy [13]. The similarity between the chromatograms obtained from extracts of Baltic and Spanish fossil resins is also worthy of note. The similarity could, in turn, be a result of diagenetic changes leading to structures of greater stability which undergo further transformation only slowly.

Nothing is known of the phytogenesis of the Spanish resin analysed. In such circumstances traits in common with other resins, e.g. chemical structure, might suggest or indicate its genesis. In this instance the similar distribution of unsaturated groups of compounds might have comparative significance. Much more analysis must be performed, however, to confirm tentative assumptions about genetic relationships between the resins under discussion.

Differences between the chemical structures of different resins, and trends in these differences, also become very apparent on detection of oxygenated compounds on the TLC chromatograms. Results from detection of α-keto and α-hydroxy acids in extracts of resins (Baltic amber, Canada balsam, and pine resin) from several types of coniferous tree are given in Table III. The spots were visualised with ammonium cerium(IV) nitrate–nitric acid as indicated in the literature [11, p.3]. The chromatograms contained many overlapping and tailing spots. Two distinct tailing spots were observed for the Baltic amber extract. For Canada balsam only one tailing spot was visible, in the region 0.97–0.48, and a single spot at R_F 0.36. This indicates that Canada balsam contains a smaller variety of less polar compounds. For pine resin the oxygenated compounds were of intermediate polarity and of little variety. The chromatogram contained one simple spot at R_F 0.74 and an elongated spot in the R_F region 0.42 to 0.11. Generally higher polarity and more varied composition was observed for the extract from the older resin – Baltic amber. This is indicative of the advanced oxygenation processes to which this resin was subjected during a long period of deposition. Aerobic conditions probably characterised part of this period, e.g. during migration of resins with water or glacier into the second bed. Oxygenation, as already mentioned, probably has a substantial effect on unsaturated compounds present in the structure of the resins. These bonds can combine with oxygen to form OH groups [13]. Under natural condition, in the presence of natural catalytic agents, other oxygen bonds can be probably formed.

Table III

Detection of α-keto acids and α-hydroxy acids in ethanol extracts of Baltic amber, Canada balsam, and pine resin

Baltic amber extract, R_F	Canada balsam extract, R_F	Pine resin extract, R_F
Tailing, 0.98–0.53	Tailing, 0.97–0.48	0.74
Tailing, 0.21–0.00	0.36	Tailing, 0.42–0.11

Retention coefficients estimated for carboxylic acids detected in chromatograms obtained from extracts of Baltic amber, Spanish resin, and pine resin are listed in Table IV. The visualisation reagent (bromocresol green–bromophenol blue–potassium permanganate [11, p.12]) enabled detection of organic acids as yellow spots on a blue background. For the Baltic amber extract the most distinct yellow spots appeared at R_F of 0.79 and near the mobile-phase front (0.95) and origin (0.21–0.00). The spot at the origin was identified as the dicarboxylic acid succinic acid, a characteristic component of succinite, by comparison of its R_F with that of a standard (R_F 0.19). Other very faint spots were also observed in the chromatogram obtained from Baltic amber extract. All spots appeared on a dark blue tail, indicative of the considerable variety of this group of compounds in the molecular phase of Baltic amber, although in relatively low quantities.

Table IV

Retention coefficients, R_F, estimated for organic acids detected in chromatograms obtained from ethanol extracts of Baltic amber, Spanish resin, and pine resin

Sample extract	R_F
Baltic amber	0.95, 0.84, 0.79, 0.69, 0.58, 0.47, 0.21, 0.08, 0.00
Spanish resin	0.93, 0.89
Pine resin	0.85, 0.75, 0.03

The chromatogram obtained from the Spanish resin extract contained fewer yellow spots than that from the Baltic amber extract, indicating lower differentiation of the acidic compounds. The acids in the Spanish resin extract were also less polar (R_F 0.93 and 0.89). These tendencies are indicative of the presence of less oxygenated material in the cretaceous resin extract than in that from the tertiary resin; this can be explained on a basis of geochemical studies. It has been stated that diagenetic transformations of fossil organic matter occur, among others, in a direction which reduces the amount of oxygen [14]. Impoverishment of oxygen bonds in the Spanish resin extract could be as a result of a natural diagenetic process more advanced than for Baltic amber. The Baltic amber extract, however, contained a generally greater variety of oxygen-containing compounds than the contemporary resins pine resin and Canada balsam. This is only an apparent contradiction, because during one of the initial stages of natural transformation a resin could undergo partial oxygenation, because of the presence of, e.g., unsaturated bonds, creating oxygen bonds. Further, sub-

sequent, transformation later reduces the oxygen content, in accordance with the tendency mentioned earlier, as the processes of diagenesis become more advanced. This hypothesis should be, however, confirmed by further analyses of a greater number of resins of different diagenetic transformation.

The phosphoric acid water solution [11, p.68] was subsequently used as a general detection reagent for comparison of the ethanol-soluble components of Baltic amber, dammar, and pine resin. The chromatograms obtained contained tailing but with a series of spots marked more distinctly. The respective retention coefficients are listed in Table 5.

Table V

R_F values and spot colour after general detection, with phosphoric acid as visualisation reagent, of chromatograms obtained from ethanol extracts of Baltic amber, dammar resin, and pine resin

Baltic amber extracts		Dammar resin extract		Pine resin extract	
R_F	Spot colour	R_F	Spot colour	R_F	Spot colour
		0.95	Brown		
0.96	Maroon	0.90	Purple		
0.93	Maroon	0.84	Brown	0.95	Brown
0.83	Purple	0.80	Brown–purple	0.91	Brown
0.77	Brown	0.77	Brownish	0.86	Brown
0.67	Purple	0.74	Brownish	0.74	Brown
0.59	Brown	0.69	Purple	0.61	Brownish
0.48	Brown	0.66	Purple	0.47	Brownish
0.41	Red	0.58	Purple	0.41–0.20	Yellow–brown
0.34–0.03	Yellow–brown	0.52	Brownish		
		0.46–0.22	Brownish		

There are large differences between the chromatograms obtained from the ethanol extracts of resins from coniferous (pine resin) and deci-duous (dammar resin) trees (Table V). The composition of the extract from the deciduous tree was more complex than that from the pine resin. The substantial difference between two contemporary resins of different origin seems to have much comparative significance. Differences between chro-matograms obtained from the soluble parts of pine resin and Baltic amber are also apparent. Use of sulphuric acid as another reagent for general vi-sualisation enabled comparison of chromatograms obtained from extracts of resins of different geological age – tertiary (Baltic amber) and cretaceous (Spanish resin) (Table VI).

Table VI

R_F values and spot colour after general detection, with sulphuric acid as visualisation reagent, of TLC chromatograms obtained from ethanol extracts of Baltic amber and Spanish resin

Sample	R_F	Spot colour
Baltic amber extract	0.97	Brown
	0.92	Purple
	0.85	Purple
	0.78	Brown
	0.67	Purple
	0.59	Brown
	Tailing, 0.50–0.00	Brown
Spanish resin extract	0.94	Brown
	0.88	Purple
	0.66	Red
	0.56	Purple
	0.42	Maroon
	0.29, 0.17, 0.11	Brown

The phytogenesis of Spanish resin is unknown. Some similarity of the chromatograms obtained from extracts of both the resins investigated might, however, suggest genetic similarity. For confirmation of this hypothesis much more evidence is undoubtedly needed.

CONCLUSIONS

The results obtained have shown the possibilities of differentiating among the chemical structures of natural resins by TLC investigation of the composition of the compound groups contained of their extracts. Dissimilarities were observed in chromatograms of extracts obtained from contemporary resins of different genesis. There were also characteristic differences between chromatograms of extracts obtained from contemporary and fossil resins. The similar group composition of some samples can be used as a basis for discussion of genetic relationships. Thus, despite the indirect method of analysis, by investigation of extracts, comparative studies of chromatograms obtained under different analytical conditions might be useful for resolving the problem of genesis and classification, especially for fossil resins frequently of unknown phytogenesis.

It should be emphasized that discussion is also possible on the basis of transformation of the chemical structure of resins by natural polymerisation, partial oxygenation, and, for fossil resins, diagenetic processes also. For this purpose the composition of groups of oxygen-containing compounds could also be diagnostic, as also could groups of unsaturated compounds.

The results presented here should, however, be regarded as preliminary, because of the limited choice of samples and the introductory nature of the work.

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