Reports

Infrared Spectra as a Means of Determining Botanical Sources of Amber

Abstract. *Comparison of infrared spectra of amber no older than Oligo-Miocene with present-day resins provides convincing evidence as to source, especially with corroboration from paleobotanical remains and knowledge of living resin producers. Spectroscopy is also useful in establishing that amber in widely separated areas in the Cretaceous and Eocene is from the same botanical source, although it may not be possible to relate these older resins to those from present-day plant populations.*

Although amber (*1*) is a fossilized resin, little attention has been given to its botanical source except in the case of the extensive and well-known Baltic deposits (*2*). The task of classifying amber has fallen to the mineralogist (*3*), who has more or less routinely characterized it chemically and physically without concern for the relation of these properties to plant source. Although a few organic compounds have been isolated from amber (*4*), rarely has their presence given the clue to the botanical source of amber. A notable exception is the isolation of cinnamic acid and styrene from Siegburgite (*5*), which relates this fossil resin to *Liquidambar*.

Amber occurs throughout the world in sizeable deposits of Middle Cretaceous to Pleistocene ages (*2*). Small amounts of resin have been reported from Jurassic strata as well as from some Carboniferous coals. The geologic time range and the geographic spread of amber deposits makes plausible the assumption that different kinds of trees may have produced the resin; this idea is likewise supported by the diversity in chemical and physical properties. Studies on the woody inclusions in the amber from the Baltic deposits (*6*) have led to the conclusion that Succinite was derived from pines. Unfortunately, these investigations have also tended to produce the preconception among laymen and scientists alike that ambers in general are derived from pines, or at least conifers. Therefore, the possibility of determining the botanical sources of ambers, not only by indications from inclusions but by chemical data that could connect them with living resin producers, is of considerable interest.

Recently, evidence has been obtained that the botanical origin of fossil resins can be determined by means of infrared spectroscopy with greater certainty than by other methods now on record (*7*). Spectra of amber were obtained in the solid state dispersed in potassium bromide pellets. The large number of absorption bands which amber shows between 4000 and 625 cm^{-1} (2.5 to 15 μ) offers a multiplicity of parameters from a single experiment which can be performed in 20 minutes. Thus it is practicable to examine a large number of samples. Spectra were run of amber from different geographic areas and geologic ages. Where sufficient material was available, a good statistical sample was run for a given area. Spectra also were made of resins from possible living amber producers.

All spectra, both from amber and modern resins, show more similarities than differences in the region 4000 to 1250 cm^{-1} (2.5 to 8 μ) where absorption bands are due to stretching and deformation vibrations of functional groups which are little affected by the intramolecular environment. At higher wavelengths, where skeletal vibrations appear, the differences are more pronounced. The identity of two infrared spectra of pure compounds can be taken as proof of the identity of the compounds. For some highly complex mixtures of heterogeneous biological materials of very high average molecular weight, such as fossil resins, two restrictions must be made: (i) only major constituents can be expected to give strong absorption bands, so that differences in minor constituents may not be apparent at all; (ii) identity of spectra can never be expected even from samples from a single specimen, but the presence of similarities, particularly in the upper "fingerprint" range, is evidence for structural similarity (not identity) of major constituents. Within these limitations the similarity of spectra of resins which are separated in time or space is the best evidence for botanical relationship short of complete chemical analysis. The similarity of the spectra of fossil and present-day resins indicates considerable stability of certain linkages through approximately 110 million years (from specimens ranging in age from the early portion of the Late Cretaceous to the present). The consistency of the entire spectrum of amber within a locality is startling, especially considering the possible variation in depositional conditions as well as subsequent exposure in outcrops. Consistency in total spectrum has been observed for nearly all Baltic specimens of the Succinite variety (*8*). Of 234 spectra of this resin, all but three are similar. Especially noteworthy is the easily recognizable absorption band of medium intensity at 1160 to 1150 cm^{-1} (8.6 to 8.7 μ), which is preceded by a more or less flat shoulder of nearly 0.5 μ width (Fig. 1). This pattern is highly suggestive of that shown in the same region by diethyl succinate (*9*), which would confirm the classical

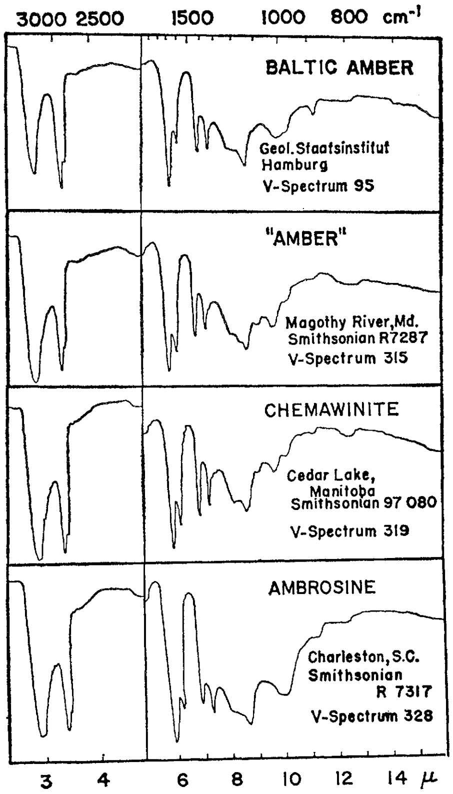

Fig. 1. Comparison of spectra of typical Eocene Baltic amber with those from Cretaceous amber from Maryland, from Charleston, South Carolina, and from Manitoba, Canada (*17*).

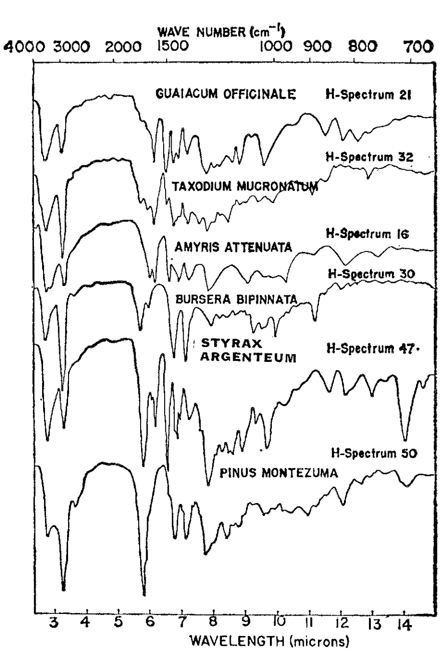

Fig. 2. Comparison of spectra from representatives of copious resin producers living in southern Mexico today.

notion that the presence of large amounts of esters of succinic acid characterizes the Baltic Succinite.

Amber samples from other localities, such as Alaska, Sicily, Burma, and Japan, show their own characteristic patterns that we have only begun to study and interpret. It is of particular interest, however, that specimens from Cretaceous deposits in Maryland, near Charleston, South Carolina, and at Cedar Lake, Manitoba (one of eleven samples) have spectra indistinguishable from the Eocene Baltic amber (Fig. 1). Amber specimens thus far examined from the Upper Cretaceous Raritan Formation at Kreischerville, Staten Island, New York, do not have the typical Baltic spectrum, although succinic acid has been reported for some specimens from this locality (*10, 11*). The spectra from Kreischerville are similar to the majority of those run from Manitoba. A variety of conifers has been described from the Kreischerville deposits and has led several paleobotanists (*11, 12*) to conclude that different kinds of trees probably produced the amber. Amber has been found intact in both pine (*13*) and *Sequoia*-type woods (*12*). A possible multiple source of the resin from both Kreischerville and Manitoba deposits may explain the difference in spectra. The most significant point, however, is the duplication of spectra from the Baltic Eocene deposits and North American Cretaceous deposits. The great similarity of entire spectra of ambers separated both geographically and by a time interval of millions of years implies that the spectral character is likely determined by the plant source.

In a detailed study of amber from the Oligo-Miocene Simojovel Formation of Chiapas, Mexico, spectra of resins from such genera as *Styrax, Amyris, Myroxylon, Guaiacum, Pistacia, Bursera, Protium, Hymenaea, Pinus, Picea, Taxodium*, which live in the area today, were examined. Each genus was clearly distinguishable, as indicated by the six representatives presented in Fig. 2. Populations of certain species, as well as closely related species within a genus, were found to have characteristic, reproducible spectra. Remarkably consistent spectra were obtained from 15 resin samples of *Hymenaea courbaril* L. from various populations in Mexico, Guatemala, British Guiana, Brazil, Venezuela, and Ecuador. Six typical spectra from this group shown in Fig. 3 display these similarities, particularly for the pairs Mexico-Guatemala, British Guiana-Brazil, and Venezuela-Ecuador. It bears mention that relatively little morphological variation occurs in this population throughout its wide range from Brazil to Mexico (including the West Indies), although several varieties of it have been recognized at the center of distribution of the genus in the Amazon region. Spectra of resins from *Bursera bipinnata* Engl. and *B. excelsa* (HBK) Engl. from different localities in Mexico also are consistently similar. Spectra of pine resins show reproducibility within some species populations, but display appreciable differences in spectra from populations where morphological variation is known to be a problem. These findings parallel the more definitive findings of Mirov (*14*) that the chemical composition of turpentines in pines is genetically controlled and may be used effectively in certain cases as a taxonomic property. Any genetic variability is mirrored not only morphologically but also in the chemistry of the turpentine. Mirov cautions that it is not to be assumed that biochemical and morphological evolution always proceed at the same rate. His data, however, increased our confidence that the chemical composition of resins could be used as a taxonomically distinct character for certain plant taxa.

The consistency in spectra found both in the resin from certain living populations and in amber from areas separated in space and time encouraged a comparison between fossil and present-day resins. Such a comparison has been successful in relating resin of *Hymenaea courbaril* L. with amber from Chiapas, Mexico. Of 29 Chiapas amber samples run, almost half have spectra closely similar to that of *Hymenaea* (Fig. 4*A*), although with some differences. The broad band at 695 to 705 cm^{-1} (14.2 to 14.4 μ), due to an unassigned skeletal frequency, has disappeared in the fossil resin; this is an expected consequence of polymerization. The sharp band at 885 to 890 cm^{-1} (11.2 to 11.3μ) is less intense in the amber than in the modern resin; since this band can be assigned to $=CH_2$ out-of-plane deformation of terminal carbon-carbon double bonds, its decrease would be an expected result of progressive oxidation by atmospheric oxygen. The attendant formation of new carbon-oxygen bonds would also account for the slight shift

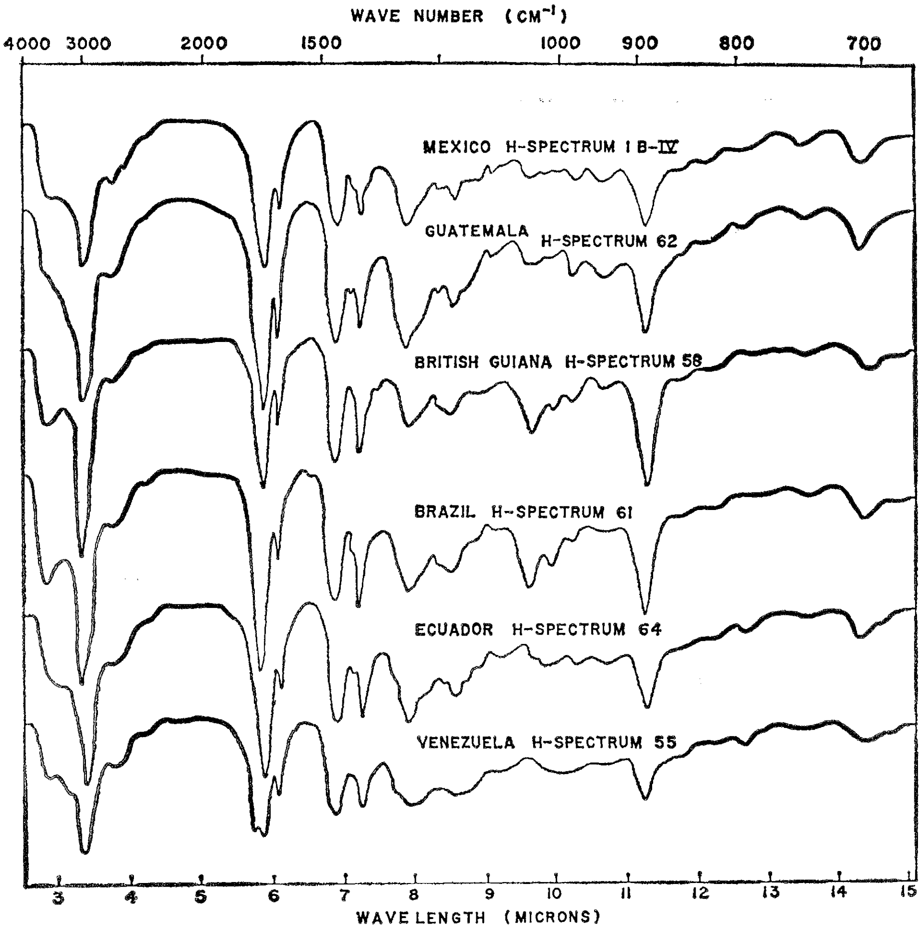

Fig. 3. Comparison of spectra of resin from *Hymenaea courbaril* L. from various localities in Mexico, Guatemala, British Guiana, Venezuela, Ecuador, and Brazil.

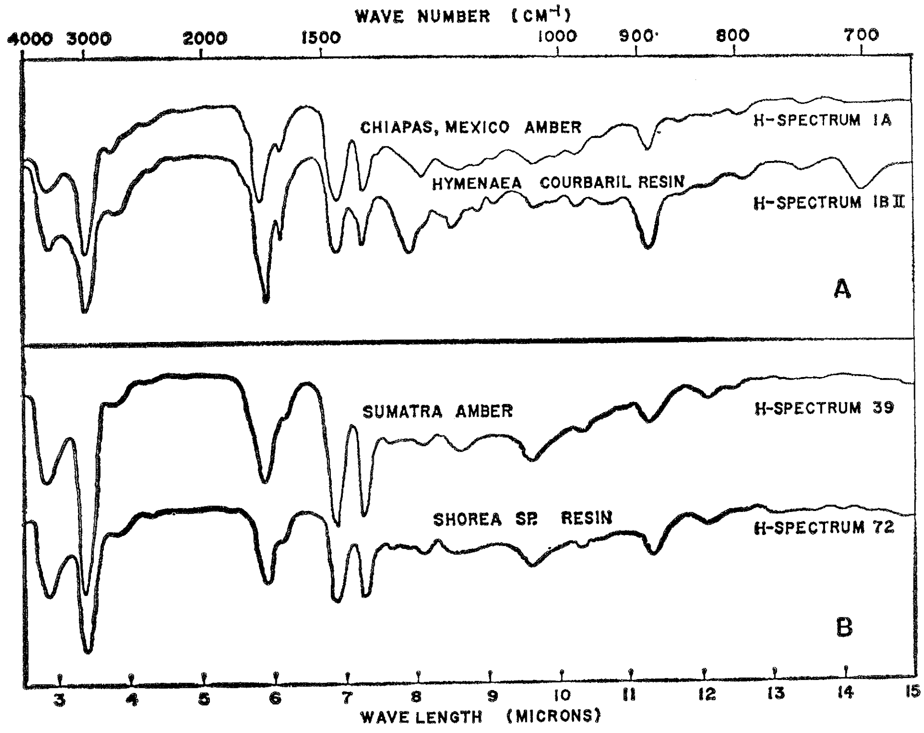

Fig. 4. (*A*) Comparison of spectra of Oligo-Miocene Chiapas amber and a resin from a Mexican population of *Hymenaea courbaril* L. (*B*) Comparison of spectra from Miocene amber from central Sumatra and a resin of *Shorea* sp. from Borneo.

of the absorption maximum near 1250 cm^{-1}, which is more often at 1265 cm^{-1} (7.9 μ) in the resin of modern *Hymenaea*, but more often at 1235 cm^{-1} (8.1 μ) in the Chiapas amber. It is likewise significant that none of the other resin producers of Mexico today, including *Pinus*, had spectra resembling the Chiapas amber (Fig. 3). Corroborative evidence for the probability that the amber was derived from ancestral *Hymenaea* populations is an apparent leaf and sepals of *Hymenaea* included in the amber from which several spectra were run. Also, both the habitat conditions and the characteristics of the secretory system of *Hymenaea courbaril* in Mexico today indicate that it is a more likely source than any of the numerous resin producers growing there today. The resin of *H. courbaril* accumulates in large quantities in the soil around these trees which grow along the coastal streams that enter estuaries in sites demonstrated (by pollen and invertebrates) to be similar to those in which the amber was deposited. Little evidence for major evolutionary change since the Oligo-Miocene is presented by flowers (*15*) as well as by several groups of insects in the amber (*16*). This apparent lack of appreciable change may well be reflected in the chemical composition of *Hymenaea* resin. Undoubtedly more detailed chemical study would show differences between the compositions of Oligo-Miocene amber and the resins of living populations. The concomitance of evidence, however, provides convincing support for the correlation of the spectra, and hence for the source of the amber.

Another example of similarity between infrared spectra of fossil and present-day resins is provided by the spectra of Miocene amber from central Sumatra and of *Shorea* sp. resin from North Borneo (Fig. 4*B*). At present there are no corroborative botanical data on this amber and only a few spectra from the living Dipterocarpaceae have been measured. Correlation of the spectra, nonetheless, is highly suggestive of a source relationship.

JEAN H. LANGENHEIM
CURT W. BECK
Radcliffe Institute for Independent Study and *Botanical Museum, Harvard University, Cambridge, Massachusetts* and *Department of Chemistry, Vassar College, Poughkeepsie, New York*

References and Notes

1. In the strict sense, as a translation of the German word *Bernstein*, the name "amber" has been limited by some workers to the Baltic variety known to the mineralogist as Succinite, but it is better used in the broad sense to denote any fossil resin. It is in this latter and wider sense that we use the term.
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17. "V-Spectrum" designates spectra made at Vassar College and "H-Spectrum" those made at Harvard University.
18. This work was supported in part by NSF grant GB 1312, and funds from the Radcliffe Institute for Independent Study.

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Uranium-Series Ages of Pacific Atoll Coral

Abstract. *The thorium-230:uranium-234 method of dating corals and oolites has been evaluated in detail for reliability, and various criteria have been established. Reliable ages for extensive coral formations of about 6000 and 120,000 years were obtained. A hiatus in the development of coral between 6000 and 120,000 years ago on the Pacific atoll of Eniwetok implies that conditions did not permit coral growth during this period. The record prior to 120,000 years ago is not clear, probably because of a lack of unaltered samples.*

Barnes *et al.* (*1*) first demonstrated the feasibility of using Th^{230}, the daughter of U^{238}, for dating marine carbonates; they showed that living corals contain several parts per million (ppm) of U^{238} almost completely free of its product, Th^{230}. Fossil coral from progressively greater depths in borings from Eniwetok yielded progressively higher Th^{230}:U^{238} ratios, approximating the equilibrium ratio at depths beyond 30 m. This discovery suggested the possibility that Pleistocene marine carbonates in the range of a few thousand to a few hundred thousand years old might be dated by this technique.

Subsequent studies (*2*), while substantiating the initial work, emphasized that recrystallization was evidence that the chemical system had not remained closed. Both this and other work (*3*) suggested that the presence of Th^{232} was evidence of possible incorporation of external Th^{230}. Thurber's report (*4*) that the dissolved uranium in sea water is enriched in U^{234} activity by 15 percent over that of its parent U^{238} not only required a revision of previous age estimates but also opened the possibility of an independent age method based on the decay of the excess U^{234}.

Comparisons between Th^{230} and C^{14} ages, although limited to samples less than 25,000 years old, provided an obvious means of establishing the validity of both methods. The relation between the concentration of Ra^{226} and that of its parent Th^{230} offered a means of checking the assumption that the system had remained closed for the past few thousand years. Our report is of a cooperative effort between Washington University and Columbia University groups to apply these approaches in evaluating Th^{230} ages obtained on carefully selected coral samples from borings made in the mid-Pacific atolls (*5*). Fanale and Schaeffer (*6*) report analyses of He:U in several of these samples as an independent means of establishing their absolute age.

The quantitative relations between the four isotopes U^{238}, U^{234}, Th^{230}, and Ra^{226} as a function of age are given in Figs. 1 and 2 (*7*). The initial ratios of U^{234}:U^{238}, Th^{230}:U^{234}, and Ra^{226}:U^{234} are taken respectively as 1.15, .00, and .09 (see below). The system is assumed to have been closed to further addition or removal of these isotopes; the equations used to generate these graphs are given by Broecker (*8*).

The measurements reported here were made as follows. Th^{230} and Th^{232} were measured by alpha-counting thin sources of separated thorium. The Washington University group used alpha-decay curve analysis and alpha spectrometry (*2, 9*); the Lamont group, alpha spectrometry (*10*). Uranium measurements were made by either fluorometry (*8, 11*) or alpha spectrometry. Ra^{226} measurements were made by alpha counting the Ra^{222} daughter (*8*); C^{14} measurement, by proportional counting of CO_2 gas (*12*). The percentage of calcite was determined by x-ray analysis.

In Table 1 the ratios of one nuclide to another are relative decay rates rather than mass ratios. Two nuclides at radioactive equilibrium will have an activity ratio of unity. The first and last samples in Table 1 are control samples: one living and one very ancient coral. Ratios observed for the living coral are consistent with previous estimates (*2, 3, 13*); they are used to establish the initial conditions listed above. As expected, the Miocene coral shows equilibrium throughout the uranium decay series. Based on the results on living coral and on the assumption that the system has remained closed, the following criteria can be used to evaluate the results on samples of unknown age.

1) The sample should contain less than a few percent calcite. As coral forms as pure aragonite, the presence of calcite indicates that recrystallization or cementation has taken place; such processes are likely to lead to violation of the closed-system assumption.

2) The uranium concentration in the

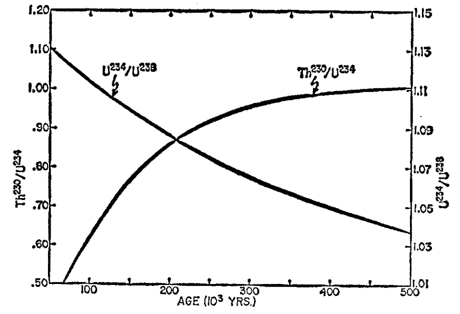

Fig. 1. Theoretical decay and growth curves for U^{234} and Th^{230} in a fossil marine coral.