common only in the mixed-habitat sites, where both adults and subadults occur. The distribution of these herbivores supports Romer's suggestion, made 40 years ago, that *Edaphosaurus* preferred swamps (*18*). The preferred habitat of juvenile *Edaphosaurus* is not yet known.

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7. Sediments are described in the works cited by Case and Romer (*6*). Additional field studies and laboratory petrography was carried out by R.T.B. (unpublished observations). Red and green mottled mudstones with small carbonate nodules identical to those at William's Ranch are discussed by T. M. Bown [*Geol. Surv. Wyo. Mem. 2* (1979)], who argues persuasively that these structureless nodules grew in the B horizon of soils in floodplains that were at least seasonally well drained.
8. Geraldine Bonebed: American Tribune New Colony Subdiv., Sect. 98, Archer Co.; Briar Creek Bonebed: Dallas Co. School Land, Sect. 32, Archer Co.; Rattlesnake Canyon: Southern Pacific RR Survey A-412, Sect. 1, Archer Co.; and West of Williams' Ranch: J. Gibbs Survey A-566, Baylor Co. Maps of Romer's collection area are available in works cited in (*6*).
9. A. S. Romer, personal communication.
10. Some sediment samples from West of William's Ranch show small lenses of cross-bedded clay clasts and reworked carbonate nodules; cross sets were no larger than 7 cm.
11. All specimens in the following institutions were examined: Museum of Comparative Zoology, Harvard University; Yale Peabody Museum, Yale University; American Museum of Natural History, New York; Smithsonian Institution, Washington, D.C.; Field Museum of Natural History, Chicago; Museum of Paleontology, University of Michigan; Museum of Paleontology, University of Kansas; Paleontology Collection, University of Texas, Austin; Collections, Department of Paleontology, University of California, Berkeley; and Paleontology Collections, Biology Department, University of California, Los Angeles.
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15. Two *Dimetrodon* femora from the Upper Godwin Creek Bonebed, Belle Plains Formation, show complete ossification at 135 mm length, 50 mm less than for all other femora. These specimens are too slender to be *Secodontosaurus* (*13*) and indicate a small adult *Dimetrodon*-like genus.
16. Because most of the size-frequency distributions were not normal and because differences in the shape of the distributions among habitats for each species were of principal interest, the distributions were subjected to a χ^2 test for k independent variables [S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), pp. 174–178]. The following sets of samples display significant departures from the hypothesis of a single common shape, at the .05 level: all *Ophiacodon*, $\chi^2(10) = 60$; all *Dimetrodon*, $\chi^2(15) = 39$; *Dimetrodon*, from West of William's Ranch and from Rattlesnake Canyon, $\chi^2(8) = 37$; *Dimetrodon*, from Geraldine Bonebed and Briar Creek, $\chi^2(6) = 35$; and all *Eryops*, $\chi^2(8) = 41$.
17. *Dimetrodon* had a dentition adapted for killing and dismembering relatively large prey. However, the genus probably did hunt semiaquatic reptiles and amphibians in the shallows and along stream and pond margins, as well as terrestrial species. Specimens of *Eryops* humeri at Harvard show deep teeth marks which match the cross-section shape of *Dimetrodon* teeth.
18. A. S. Romer, *Geol. Soc. Am. Bull.* **46**, 1597 (1935).
19. I thank the curators at the institutions enumerated in (*10*) for access to the collections; most especially, I am grateful to the late A. S. Romer for his interest in this project, though some of the results may be contrary to some of his conclusions; when shown the ossification sequence for Briar Creek, Romer commented that ". . . yep, all those *natalis* could be juveniles."

6 April 1982

Carbon Functionalities in Amber

Abstract. *High-resolution nuclear magnetic resonance spectra of the carbon nuclei in powdered amber, obtained by using the techniques of magic angle spinning and cross polarization, provide detailed information about the types of carbon functionalities. The entire spectrum of Baltic amber (succinite) is identical for several samples. Baltic amber shows minor differences from Sicilian amber and drastic differences from Burmese, Romanian, and Bohemian ambers.*

Because of its use over several millennia as a raw material for decorative objects and its origin from ancient plant materials, amber has been of interest to the archeologist (*1*) and the paleobotanist (*2*) alike. Knowledge of the chemical structure of amber would be of use both in associating a given object with its geographic origin and in determining the botanical identity of the tree from which the source resin of amber came. Efforts to obtain a detailed description of the chemical structure of amber have been thwarted by its noncrystallinity and poor solubility. Thus are eliminated the two most general structural tools, x-ray crystallography and solution phase nuclear magnetic resonance (NMR) spectroscopy (*3*). Infrared spectroscopy of powdered amber has provided the most useful structural information to date and has been particularly successful as a diagnostic for Baltic amber (succinite) (*4*). The analytical problems with amber are very similar to those with coal. Both materials have a complex organic structure and are poorly soluble in spectroscopic solvents. Recently developed methods for obtaining high-resolution NMR spectra of solids (*5*) have been exploited very successfully in the analysis of coal and related materials (*6*). Consequently, we examined a variety of ambers by this technique, and we report here a detailed description of the chemical types of carbon present.

Figure 1 shows a typical spectrum of the ^{13}C resonances of a powdered sample (0.5 g) of Baltic amber, taken with magic angle spinning and cross polarization (CPMAS) and averaged over multiple scans (*7*). Three other Baltic samples of various colors and opacities had essentially identical spectra. Whereas the infrared spectrum was diagnostically useful primarily in the C–O stretching region (1250 to 1100 cm^{-1}), the ^{13}C spectrum is diagnostic over the entire range of resonances. Compared to analogous spectra of coal, those of amber have remarkable fine structure. Either amber contains fewer free electrons that would broaden the resonances, or coal contains a much wider distribution of functional groups that would lead to an almost featureless pattern.

Because the amber spectra contain considerable fine structure, a relatively detailed description of the carbon functionalities is possible. The resonances from approximately 0 to 100 ppm are due to saturated carbons and comprise about 86 percent of the total for the Baltic ambers. Those from 100 to 160 ppm are due to unsaturated carbons (alkenic and aromatic) and comprise 11 percent of the total. For comparison, the saturated:unsaturated ratio for coal is about 50:50. Finally, the resonances above 160 ppm in frequency are from carbonyl groups and comprise 3 percent of the total. Integrated intensities in CPMAS spectra include uncertainties due to the differing efficiencies of polarization transfer from the proton pool to the various carbon nuclei. In addition, for heterogeneous samples such as coal, wood, or amber the protons may have different relaxation times in different regions of the sample. The integrations should be valid, however, for detecting and comparing trends among related samples, as in the present study. The chemical shifts are reliable to 1 ppm.

The carbonyl resonances in Fig. 1 are entirely from acids and their derivatives. There are no aldehydes or ketones, which would resonate above 190 ppm. The relative amounts of esters (CO_2R), acids (CO_2H), and (probably) ionized acids (CO_2^-) are represented by the distinct peaks respectively at 173, 180, and 187 ppm. It is clear that most of the acid functions are present as esters. Succinic

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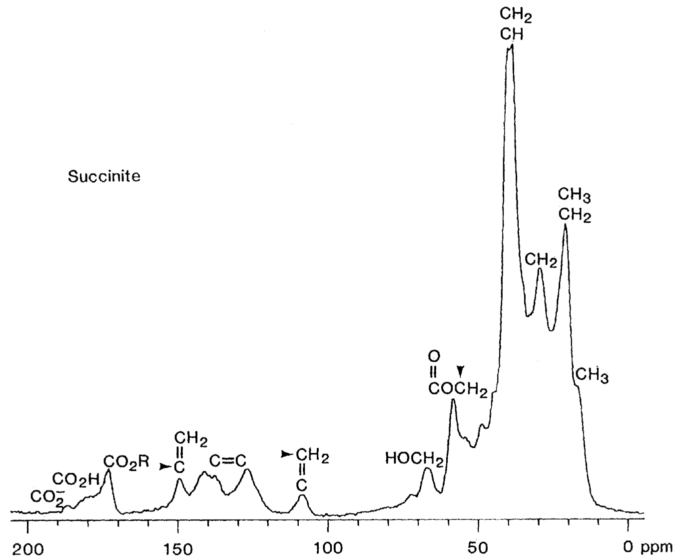

Fig. 1. Carbon-13 nuclear magnetic resonance spectrum of a powdered sample of Baltic amber from Samland (Geologisches Staatsinstitut, Hamburg; C. W. Beck inventory No. B-2), taken at 37.74 MHz with cross polarization and magic angle spinning for 55,000 scans at room temperature on a Nicolet NT-150 spectrometer.

acid itself resonates at 181 ppm and could provide less than 1 percent of the total.

The well-resolved peak at 108 ppm in the unsaturated region is from $C{=}CH_2$ (*exo*-methylene) groups. Beck and coworkers (*4*) originally inferred the presence of this group from the 885 cm^{-1} peak in the infrared. The peak at 149 ppm in Fig. 1 is also from the $C{=}CH_2$ group, in which the resonating carbon must be quaternary to fall at this high frequency. The sum of these two resonances provides about 2 percent of the total. The resonances at 127 ppm are either from internal alkenic carbons (C–CH=CH–C) or from various types of aromatic carbons. The broad pair of peaks centered around 140 ppm is from the internal unsaturated carbon of vinyl or isopropenyl groups or from aromatic carbons attached to alkyl or alkenyl groups. There are noticeable lacunae at 160 ppm, where phenolic carbons would resonate, and at 133 ppm, where certain naphthalene and anthracene carbons would resonate.

Within the saturated region, the portion from 0 to about 50 ppm comes from groups that are entirely hydrocarbon or are functionalized only with carbonyl. Above 50 ppm are carbons next to oxygen. The shoulder at 17 ppm is from most of the methyl groups. The peak at 21 ppm is from methylene groups in ethyl or from certain methyl groups (next to carbonyl, quaternary, or non-*gauche* methinyl carbons). The peak at 30 ppm is from standard methylene groups flanked by two other methylene groups, which are common in terpenes, and from methylene groups next to carbonyl or phenyl carbons. Finally, the large peak at 39 ppm is from methinyl carbons in general and methylene carbons flanked by at least one tertiary or quaternary carbon.

The largest peak in the region associated with oxygen substitution is at 58 ppm. This position is characteristic of the methylene carbon in the ethyl group of ethyl esters. The methyl carbon in methyl esters may give rise to the shoulder at 49 ppm. Methylene carbons next to OH give rise to the peak at 67 ppm, and methinyl carbons next to OH or methylene carbons next to OC (ethers) give rise to the shoulder at 72 ppm.

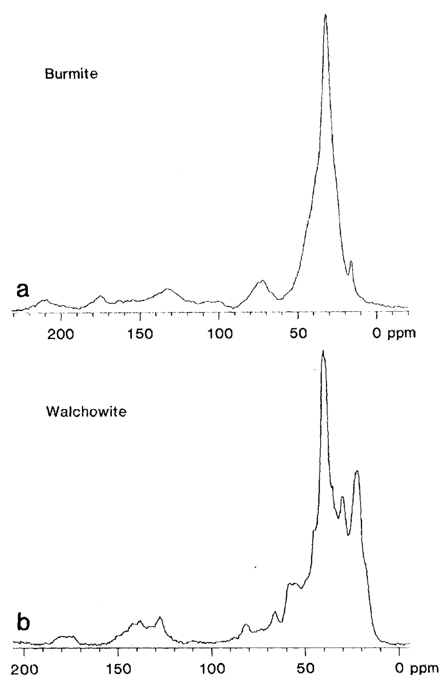

Fig. 2. (a) Carbon-13 NMR spectrum (15,000 scans) of powdered burmite from Mangotaimaw Hill, Burma (Musée National d'Histoire Naturelle, Paris; inventory No. 101.604). (b) Carbon-13 NMR spectrum (40,000 scans) of powdered walchowite from Boskovice, Moravia (New York Museum of Natural History; inventory No. 17300). See legend to Fig. 1 for further information on spectral conditions.

The ^{13}C spectrum provides not only a detailed census of the carbon functional groups but also a means of distinguishing different types of amber. We were provided with several other ambers or amber-like materials, which corresponded to the groups used previously in Beck's classification of ambers based on computer analysis of the infrared spectrum (*8*). The spectrum of beckerite is almost identical to that of succinite. Indeed, beckerite is considered to be a contaminated variety of Baltic amber and is found in the same region. A sample of simetite (Sicilian amber) has a saturated:unsaturated:carbonyl triple ratio of 87:9:4, with functional groups basically similar to those illustrated in Fig. 1. A new peak at 35 ppm and much larger CO_2H and CO_2^- components (compared with CO_2R) are the major differences. These close similarities suggest a common or similar paleobotanical source.

Amber from Burma (burmite) shows a remarkably different spectrum (Fig. 2a). The triple ratio of 83.5 : 11 : (3.5 + 2) indicates less saturated material. Particularly significant is the new peak at 210 ppm from ketonic carbons, representing 2 percent of the total. The carbonyl component of the triple ratio is broken down into carboxyl (3.5) and ketonic (2) components. The prominent Baltic peaks at 20 and 39 ppm are lacking in burmite, as is the ester carbon at 58 ppm. Romanian amber (schraufite) also shows considerable differences, again with a ketonic contribution [triple ratio 85.5 : 9.5 : (3 + 2)]. Of particular interest in schraufite is the complete lack of the resonances at 108 and 149 ppm from the *exo*-methylene group. A sample of walchowite (Bohemian amber) has significantly reduced *exo*-methylene peaks (Fig. 2b). Additional differences between walchowite and succinite are new peaks at 54 and 83 ppm, probably from more highly branched oxygen functions, a higher proportion of CO_2H compared with CO_2R, and a triple ratio of 90.5 : 8 : 1.5 (much less carbonyl).

Thus CPMAS NMR spectroscopy provides the most detailed description of the chemical constitution of amber yet achieved. Our sampling of four Baltic ambers uniformly produced the same spectrum, which is characterized by a preponderance of ester resonances in the carbonyl region, a large proportion of *exo*-methylene resonances in the unsaturated region, ester ethoxy resonances in the *O*-alkyl region, and three main types of saturated hydrocarbon resonances. The close relation of beckerite to succinite and the only slightly more distant relation of simetite are apparent in their spectra. Large differences are seen in other ambers (burmite, schraufite, walchowite), including appearance of ketonic resonance, loss of *exo*-methylene resonances, and increase of acid and ionized acid carbonyls at the expense of esters. We hope that these newly established constitutional differences can be exploited not only as a diagnostic for geographic sources but also as an aid in the identification of the paleobotanical origin of amber through comparison with spectra of modern resins.

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Spiroplasmas: Diversity of Arthropod Reservoirs and Host-Parasite Relationships

Abstract. *Spiroplasmas were found in 11 new insect hosts representing four orders: Hymenoptera, Hemiptera, Diptera, and Coleoptera. Three of the new spiroplasmas were serologically distinct from all existing groups or subgroups. A spiroplasma that infects digestive tracts of Colorado potato beetles may be transmitted to uninfected insects that feed on contaminated plants. This simple type of spiroplasma-insect relationship may explain a growing list of isolations of spiroplasmas and other wall-less prokaryotes from plant surfaces.*

Although spiroplasmas were first envisioned (*1*) in 1961 and gained recognition (*2*) as a distinct microbial group in 1973, until 1975 only three such organisms were known. Two of these spiroplasmas, the agent of citrus stubborn disease (*3*) and the agent of corn stunt disease (*4*), cause economically important plant diseases that are transmitted by leafhoppers (*5*). A third spiroplasma causes the elimination of male progeny from broods of infected females of certain Neotropical species of *Drosophila* and is called the sex ratio organism (*1, 6*). The suckling mouse cataract spiroplasmas (*Spiroplasma mirum*) (*7*), although originally isolated from rabbit ticks in 1964 (*8*) and long thought to be a virus, was eventually recognized as a spiroplasma (*9, 10*). The discovery of a spiroplasma in the honey bee (*11*) first focused attention on flowers and plant surfaces as possible sites of insect-to-insect transmission because the seasonal occurrence of the organism in the hemolymph of bees was synchronized with the spring nectar flow. Flower and plant surfaces have since proved to be rich sources of spiroplasmas and other wall-less prokaryotes (*12, 13*). On the basis of my findings, it seems likely that many of these organisms are inhabitants of the intestines of insects and are transmitted from insect to insect by contamination of plant surfaces.

The insects were collected at the USDA Agricultural Research Center in Beltsville, Maryland (*14*). Honey bees used in these experiments were from colonies maintained at the Bioenvironmental Bee Laboratory. Many insect species were examined for spiroplasmas (*15*) (Table 1).

Hemolymph and digestive tract fluids were monitored for spiroplasmas by phase-contrast or dark-field microscopy at ×1000. Hemolymph samples were taken by puncturing intersegmental membranes at the base of the prothoracic legs with fine glass capillary tubes. Dissected digestive tracts were rinsed in three changes of sterile 0.9 percent saline solution and then transferred to Singh's mosquito tissue culture medium (SM-1) (*16*). Segments 2 to 3 mm in length from the anterior, middle, and posterior portions of the midguts were compressed between slides and cover slips and examined microscopically. Specimens were prepared for electron microscopy as described earlier (*17*). Transmission experiments were performed by feeding or injecting cultured organisms suspended in medium. Spiroplasmas that did not grow in SM-1 or in other media were transmitted to noninfected insects by injection or by feeding hemolymph or filtered (0.45-μm pore) gut suspensions from infected insects.

Antiserums (*18*) produced against spiroplasmas representing serogroups (*19*) I-1, I-2, I-3, I-4, III, IV, V, and VI (*20*) were used in serological deformation tests (*21*) to type cultivated spiroplasmas from the bumble bee (BI-1), wasp (MQ-1), and syrphid fly (EA-1) and to type the Colorado potato beetle spiroplasma (CPBS) collected from intestines of infected beetles.

Spiroplasmas seen in the hemolymph of five species of Hemiptera (Table 1) have not as yet been classified serologically because they failed to grow in culture media [M1A (*22*), SP-4 (*10*), or SM-1 (*11*)] that support growth of other spiroplasmas. Attempts to infect honey bees with hemipteran spiroplasmas also failed. It is possibly significant that four of the five hemipterans with spiroplasmas in their hemolymph were predators. The fifth hemipteran, *Lygus lineolaris* (tarnished plant bug) is a classical pest of a wide variety of crops and wild plants.

The CPBS was maintained in the laboratory by placing field-collected adults and larvae in screen cages 30 by 30 by 30 cm and supplying them with bouquets of potato leaves or tomato plants. Infected beetles could be identified by microscopic examination of the dark-brown regurgitated fluids of either larvae or adults or by examination of their semisolid fecal pellets. Pathogenicity of the CPBS was assessed by comparing survivals of 30 newly hatched larvae fed individually with suspensions of 10^8 organisms per milliliter of SM-1 medium with larvae fed medium only. After 24 hours, regurgitated gut fluid was examined microscopically for spiroplasmas. Larvae were then placed on young tomato plants in a greenhouse held at 30° to 35°C. Survival of test and control insects was compared after 2 weeks, at which time most larvae had pupated.

Adult Colorado potato beetles that had emerged from their overwintering sites in the ground were noticed on the leaves of young potato plants during the last week in May 1981. Twenty-five adult beetles were examined, and 76 percent were found to harbor spiroplasmas. These first estimates of infection incidence were probably low because spiroplasmas were initially recognized by their size, helicity, and characteristic

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