A New Molecular Type of Amber for Burma (Myanmar)[1]

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Abstract: Although all previously tested amber from Burma (Myanmar) belongs to NMR amber Group A, for the first time samples are reported with the characteristics of NMR Group B. Whereas Group A has a conifer source, Group B has angiosperms as its source. Thus, two very different types of ancient trees produced resins that led to amber from Burmese Cretaceous forests.

Key Words: Amber, Burma, burmite, Cretaceous, Myanmar, nuclear magnetic resonance spectroscopy

Introduction

Amber, or fossilized resinous plant exudate, has a broad spatiotemporal distribution (Santiago-Blay and Lambert 2007, 2017) that includes Southeast Asia. According to the review by Zherikhin and Ross (2000), Burmese amber, known locally at least from 200 BC, was first reported in the West by the Jesuit scholar and missionary Álvaro Semedo in 1613. The Scottish physicist Sir David Brewster recognized and described Burmese amber early in his career (Brewster 1814, 1832, 1835). The Gdańsk (Danzig) pharmacist Otto Helm observed characteristic differences between Burmese amber and Baltic amber and gave the former the mineralogical name burmite in 1894. Amber lacks a repeating, crystalline structure, so strictly speaking burmite is a mineraloid like obsidian and pearl rather than a mineral.

Amber from Burma comes primarily from several localities in the northern state of Kachin, particularly the Hukawng Valley (Figure 1). British Infantry Capt. S. F. Hannay visited Burmese Hukawng amber mines in 1835-6 and returned in 1837 with Capt. R. B. Pemberton, who summarized Hannay's travels and provided broad descriptions of the mining area (Pemberton 1837). Griffith (1847) developed the earliest stratigraphy of a Burmese amber site. In 1906, Laufer reported that amber from Burma had been used in Chinese sculptures and had been the object of extensive trade through the centuries, perhaps as early as the first century AD. The 1886 annexation of Burma to British India markedly

increased the supply of amber to commercial utilization and scientific interests (Noetling 1892, 1893).

Figure 1. Map of Burma (Myanmar) with the Hukawng Valley shown in the insert with five important mining sites of burmite.

The geological age of Burmese amber was controversial during the 20th century. Although the consensus favored a Cretaceous age, some workers preferred a Tertiary age. Domestic unrest in much of Burma in 1936 closed down the mines and prevented any resolution of this question. Their reopening in 1999 permitted the question to be addressed again. Cruickshank and Ko (2003) reasoned from the "preponderance of the evidence" that amber specifically from the Noije Bum Village in the Hukawng Valley, Kachin State, is from the Upper Albian of the Cretaceous (ca. 100-107 Ma). Shi et al. (2012) narrowed the range considerable by means of $^{206}Pb/^{238}U$ dating to 98.79±0.62, just into the earliest Cenomanian and in either case on the border between the Upper and the Lower Cretaceous.

Since the 1990s, increased attention has been paid to Burmese amber, owing to the realization that potentially important inclusions have been entombed there for 100 million of years (Grimaldi et al. 2002, Rasnitsyn and Ross 1998, Ross and York 2000, Ross et al. 2010). Such observations have resulted in an explosion of scientific research and papers, which Andrew J. Ross of the National Museums Scotland in Edinburgh has meticulously collected and distributed free of charge to those interested (e.g., Ross 2018, 2019).

The list in Table 1 and partially illustrated in Figure 1 represents what we believe is a fairly complete list of the published locations of amber mines in Burma reported in the scientific literature. All latitudes given are north (N), and longitudes are east (E). Fractions of coordinates often are reported in angular coordinates (DMS: degrees, °; minutes, '; seconds, "), but we have converted them to decimals. In some cases, no coordinates are given in the original papers. In such situations, we have used the coordinates of the listed, nearby localities. In all cases, the coordinates represent the closest approximation available to us for the true location of the mines. Note that all reported amber sites are located in Burma's central valley that runs north to south and is relatively close to the largest rivers of the country, the Ayeyarwady (previously known as Irrawaddy) and its primary tributary, the Chindwinn.

Table 1. Reported mining sites of burmite.

Locality	Reference
North Myanmar Region (Kachin State)	
Khanjamaw, Hukawng Valley (26.25833°, 96.56028°)	Zherikhin and Ross (2000)
Ladummaw, Hukawng Valley (26.18861°, 96.48°)	Zherikhin and Ross (2000)
Lajamaw, Hukawng Valley (26.23333°, 96.46667°)	Zherikhin and Ross (2000)
Noije Bum, Hukawng Valley (26.247°, 96.5556°)	Cruickshank and Ko (2003)

Tanai/Danai, Hukawng Valley (26.3593°, 96.7109°)	Cruickshank and Ko (2003)
Central Myanmar	
Sagaing Region (Mawlaik District, formerly Upper Chindwin District (Mawlaik town 94.4028°)	Zherikhin and Ross (2000)
Schwebo District (22.574, 95.6695)	Zherikhin and Ross (2000)
Thayetmyo (Thayet District, Magway Region) (Thayet town, 19.3228°, 95.1797°)	Zherikhin and Ross (2000)
Yenangyat (Yenangyaung) (20.467°, 94.883°)	Zherikhin and Ross (2000)
Southern Myanmar	
Pegu District (17.5°, 96.5°)	Zherikhin and Ross (2000)
Tilin (21.6961°, 94.0977°)	Cruickshank and Ko (2003)

For decades, the scientific study of amber has found itself operating amidst commercial interests of buyers and sellers and the need for gainful employment of the local residents, who often mine in dangerous conditions, at times involving child labor. In the case of Burma, the war-prone environment in Kachin State in the north, where most of the reported commercial amber mines are located, makes extraction a perilous activity (Gammon 2019).

We have examined 15 samples from Burmese mines to determine their molecular structure and to infer paleobotanical origins by nuclear magnetic resonance (NMR) spectroscopy and have discovered that there is a dichotomy within these sources.

Methods

Fifteen samples of amber from Burma were subjected to analysis by NMR spectroscopy. Two (numbers 1767 and 1768 in the collection at Trinity University) were provided by Larry Smith from the Hukawng Valley, Tanaing Township, Myitkyina District, Kachin State; three samples from this collection (1735-1737) plus three others not studied at Trinity University were obtained by co-author Jonna M. Karlberg from the Priwan Lohitsean collection, Cleopatra Gems, Chiang Rai, Thailand), six (154, 276, 375, 376, 422, and 441) came from George O. Poinar Jr. (Department of Integrative Biology, Oregon State University, Corvallis, Oregon); and one (7) was provided by the late Curt Beck (Amber Research Laboratory, Vassar College, Poughkeepsie, New York). NMR spectroscopy, along with mass spectrometry, has proved to be the best technique for analyzing the molecular structure of amber (Lambert et al. 2008). We carried

out two different NMR experiments. (1) Observation of natural-abundance carbon-13 (^{13}C) nuclei of powdered, solid samples with full decoupling of carbon from hydrogen (with removal of the scalar coupling interactions between these nuclei) was carried out only at Northwestern University for nine of the samples. Three further samples (Karlberg samples 1, 3, and 5) were analyzed only at Lund University for ^{13}C nuclei with the TOSS sequence to minimize spinning sidebands. Karlberg samples 2, 4, and 6 (corresponding to Trinity University samples 1735-1737) were analyzed by ^{13}C NMR spectroscopy at both Lund and Northwestern. By examination of the bulk, the analysis is assured to characterize the entire sample. (2) Standard one-dimensional (1D) observation of hydrogen (proton, ^{1}H) nuclei in solution state, with deuterated chloroform (CDCl$_3$) as the solvent, was carried out at Trinity University for six samples. Some of the Poinar samples analyzed for ^{13}C at Northwestern University in the 1980s and 1990s were not available, and the Beck sample 7 was insoluble. Examination of the solution phase may involve some loss of material due to partial insolubility. Proton NMR spectra can provide distinctions sometimes not apparent from ^{13}C spectra (Lambert et al. 2012). Normally, a sample of ca. 100 mg was powdered in a mortar to fine grains for the ^{13}C experiments and then dissolved in chloroform for the ^{1}H experiments.

Proton spectra were obtained at 500 MHz on a Varian Inova-500 spectrometer at room temperature without spinning. Spectra were referenced in CDCl$_3$ to TMS at δ 0.0. Typical 1D parameters were as follows: spectral width 12,000 Hz, pulse width 60°, delay time 1.0 s, acquisition time 1.0 s, and scan number 4. Solid state ^{13}C data were recorded on a 400 MHz Varian NMR System with a 5 mm T3 PENCIL probe or on a 400 MHz Bruker Avance III HD NMR Spectrometer with a 4 mm HX probe. The magic angle spinning rate was set to 5000 Hz. The cross polarization (CP) pulse sequence was used for normal proton decoupling on both spectrometers. For interrupted decoupling (dipolar dephasing), a 50 μs (Varian) or a 48 μs (Bruker) delay was applied in the ^{1}H channel just before the 180° pulse in the ^{13}C channel. We used adamantane (Varian) or glycine (Bruker) to adjust the Hartmann-Hahn matching condition for normal CP experiments and to adjust the observation pulse and the delay time for dipolar dephasing. A typical parameter set was as follows: spectrum frequency 100.544 MHz (Varian) or 100.524 MHz (Bruker), spectral width 296 ppm, pulse width 3.4 μs for the 90° pulse for both ^{1}H and ^{13}C (Varian) or 2.5 μs for ^{1}H and 4.0 μs for ^{13}C (Bruker), pre-delay time 5 s, contact time 5 ms, acquisition time 50 ms, scan number 256, carrier frequency 110 ppm, and a ramped pulse with 83 Watts used in the ^{1}H channel during contact time. Solid state ^{13}C spectra were referenced to an external adamantane peak at δ 38.3 (Varian) or to an external glycine methylene peak at δ 43.4 (Bruker) and were converted to tetramethylsilane at δ 0.0.

Results and Discussion

Sample 1767 was an inclusion-free cutting from a sample that contained a single scorpion and had the shape of a rectangular cube, implying heating and shaping. Sample 1768 was an inclusion-free cutting from a sample containing two scorpion tails. The ^{13}C spectrum of sample 1768 is displayed in Figure 2, and the ^{1}H spectrum of 1768 in Figure 3. Most peaks in the ranges δ 75-90 and 175-190 in this and other ^{13}C spectra herein are spinning sidebands generated by the rapid rotation and are to be ignored. The peak at δ 7.3 in this and other ^{1}H spectra herein is from the impurity $CHCl_3$ in the $CDCl_3$ solvent, and the peak at δ 0.0 is from the standard, tetramethylsilane (TMS).
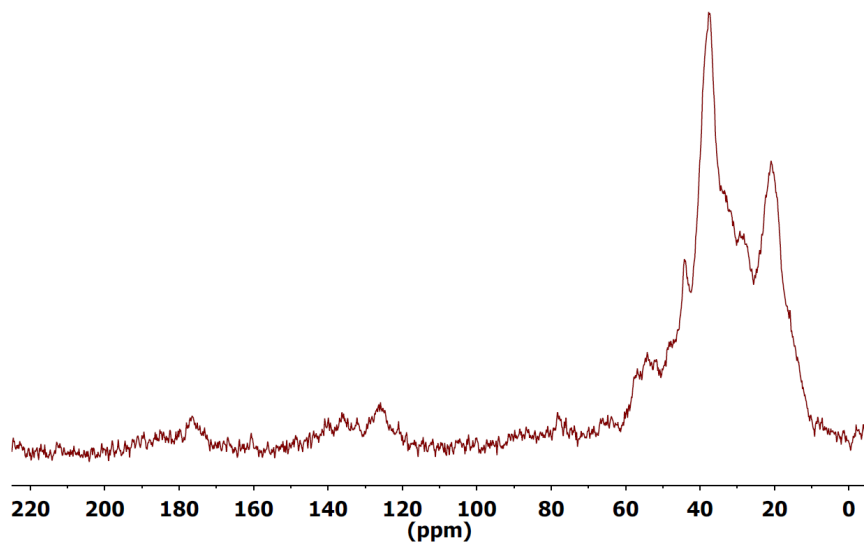
Figure 2. The 75 MHz solid state ^{13}C spectrum recorded at Northwestern University of sample 1768, provided by Larry B. Smith.
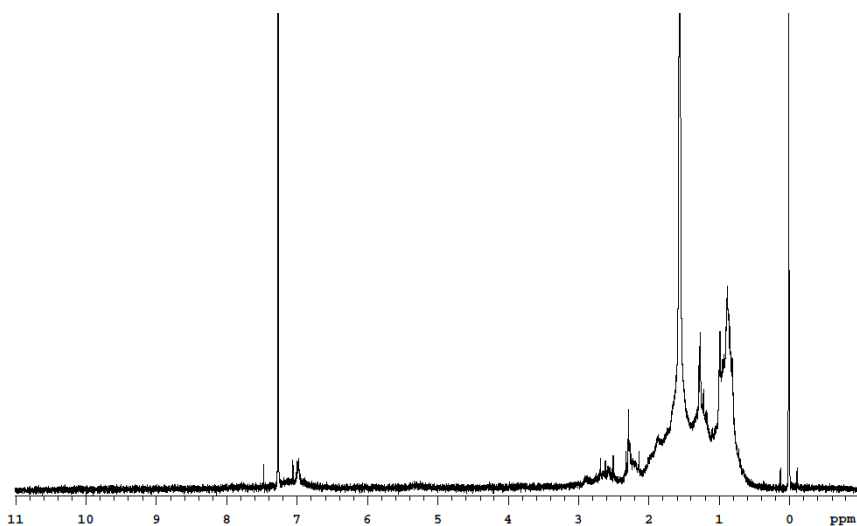
Figure 3. The 500 MHz ^{1}H spectrum of sample 1768 in CDCl$_3$, recorded at Trinity University.

Six samples from the collection of Priwan Lohitsean were examined by ^{13}C NMR spectroscopy, and two of these additionally were analyzed by ^{1}H NMR spectroscopy. These samples had been polished, but otherwise were untreated. None contained biological inclusions. Colors of the samples varied from yellow to orange, red, brown, and black. The ^{13}C spectra of five of these samples are given as an overlay in Figure 4. The ^{1}H spectrum of sample 6 (1736) is displayed in Figure 5.
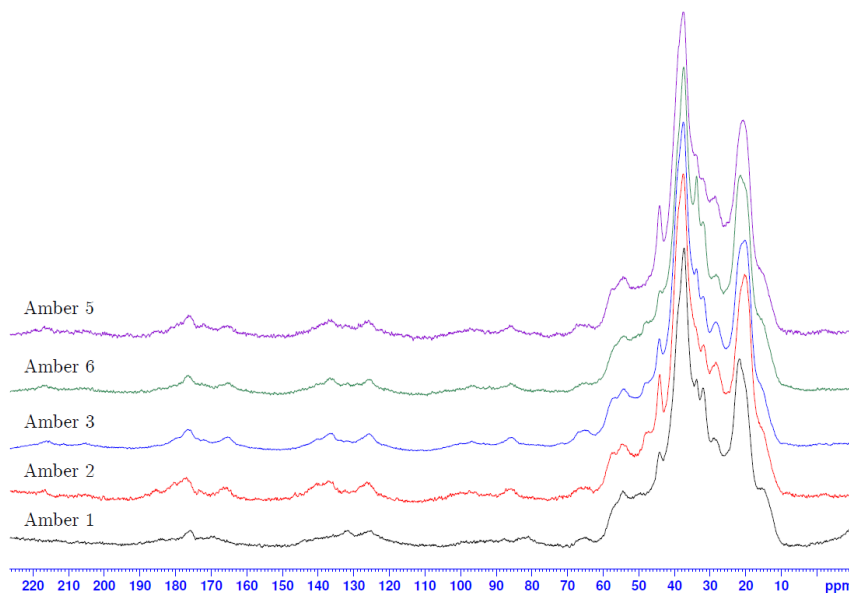
Figure 4. The 75 MHz ^{13}C spectra recorded at Lund University of samples from Tanai Township, Kachin State, Burma, provided by Priwan Lohitsean. Sample 6 is the same as Trinity University sample 1736.
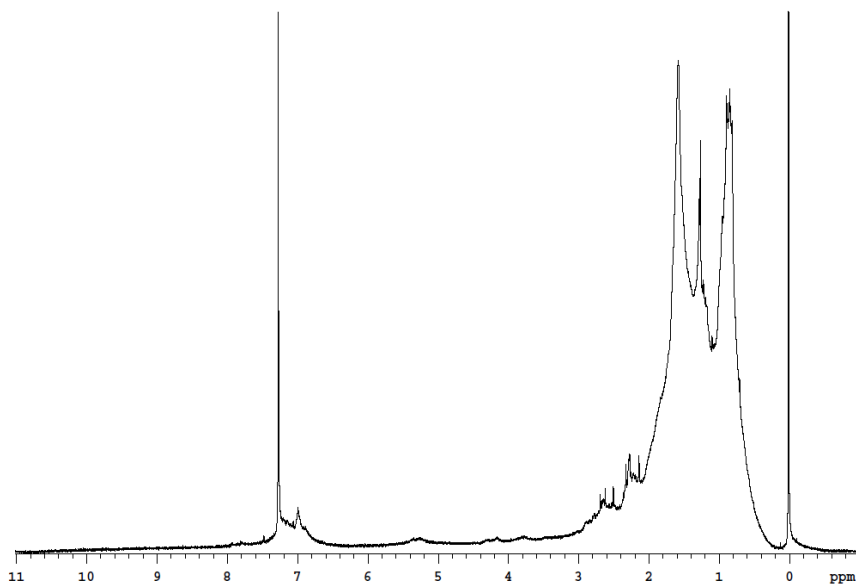
Figure 5. The 500 MHz ^{1}H spectrum in CDCl$_3$ recorded at Trinity University of sample 1736, provided by Priwan Lohitsean.

The spectra in Figures 2-5 are typical for Group A ambers, a grouping developed from ^{13}C NMR observations on our collection (Lambert et al. 1988, 2008). These materials are found widely in Europe, Asia, North America (north of Mexico), Australia, and New Zealand. In the southern hemisphere Group A amber almost certainly was derived from conifer species from the Araucariaceae (Lambert et al. 1993). The ancient botanical source in the northern hemisphere is less certain. If not Araucariaceae, the family still was coniferous and closely related, such as Cupressaceae or Pinaceae (Poinar et al. 2004). Group A amber is found in Asia from Siberia to China, Japan, Southeast Asia, South Asia, and Southwest Asia. Figure 6 shows the ^{13}C spectrum of a typical Group A amber from Japan (sample 1646, Kōsei-chō, Shiga Prefecture) (Lambert et al. 2015). The spectra in Figures 2, 4, and 6 are characterized by a dominant peak at about δ 38, another large peak at about δ 22, a definite peak at δ 55 (all from saturated carbons), a broad series of peaks in the range δ 120-140 from unsaturated (alkenic) carbons and a peak at δ 185 from a carbonyl carbon (amidst the high frequency spinning sideband). The ^{1}H spectra for Group A reliably and diagnostically contain three groupings of peaks at about δ 0.8, 1.3, and 1.5, as exemplified in Figures 3 and 5 and compared to the Group A spectrum of sample 1641 from Kobe, Hyōgo Prefecture, Japan (Figure 7) (Lambert et al. 2015).
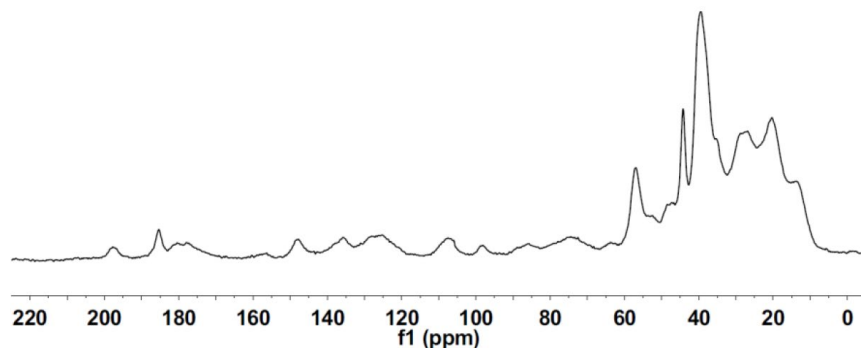

Figure 6. The 75 MHz ^{13}C spectra recorded at Northwestern University of sample 1646 from Kōsei-chō, Shiga Prefecture, Japan (Figure 15 in Lambert et al. 2015).
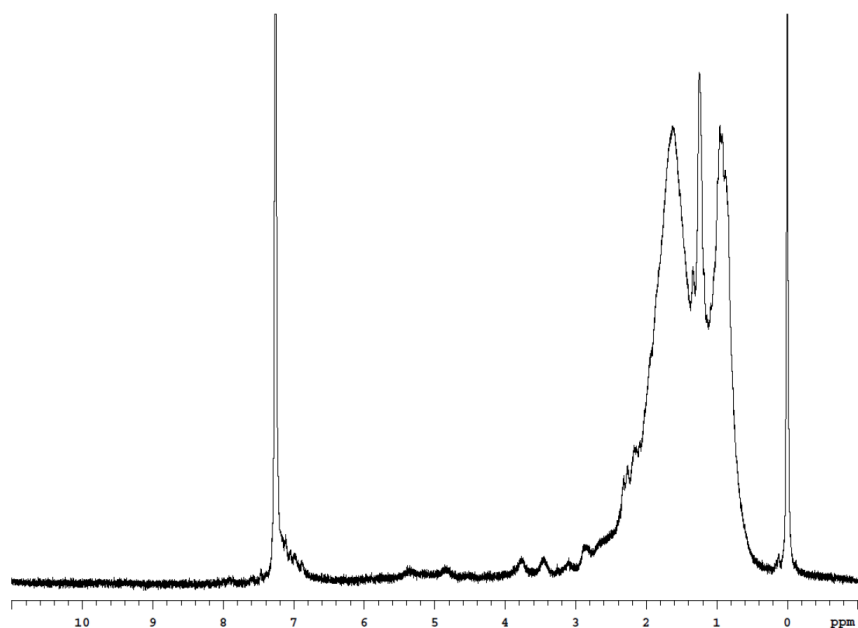
Figure 7. The 500 MHz ^{1}H spectrum of sample 1641 recorded at Trinity University from Kobe, Hyōgo Prefecture, Japan in CDCl${}_3$ (Figure 16 in Lambert et al. 2015).

One sample from the Priwan Lohitsean collection produced a slightly different ^{13}C spectrum. This sample, originally listed simply as amber sample 4 by co-author Karlberg, now carries the number 1735 in the Trinity University series. Its ^{13}C spectrum, as recorded at Lund University, is given in Figure 8. Its differences from the spectra of Group A ambers are not large but were sufficient to be singled out by Karlberg. The alkenic resonances at δ 120-140 are stronger than those in Figures 2, 4, and 6. There is a strong new peak at δ 40 in addition to that at δ 38, and a small, distinct peak has arisen at δ 59. The carbonyl peak is absent. These characteristics are suggestive of amber from Group B (Lambert et al. 2008). This grouping was discovered first in amber from the state of Arkansas in the United States (Lambert et al. 1990) and later also found in amber from Australia (Lambert et al. 1993). Whereas Group A ambers derived from conifer sources, Group B ambers came from flowering plants or angiosperms, in particular families such as the Dipterocarpaceae (Lambert et al. 2013). Although Group B ambers have been found commonly in Indonesia and less commonly in Australia, Papua New Guinea, India, Japan, and a few states of the United States, in particular Arkansas, they have not been found previously on the mainland of Southeast Asia. Consequently, further evidence was required for the conclusion that sample 1735 was a member of Group B.

The solid state ^{13}C spectrum was independently recorded at Northwestern University (Figure 9). The spectra in Figures 8 and 9 are essentially identical, although the Northwestern spectrum is weaker because of the use of fewer transients. The ^{13}C spectrum of a definite Group B sample, in this case blue amber 1418 from Kuala Tungkal, Sumatra, Indonesia, and provided by Mohammad Iskandar bin Marzuki, is given in Figure 10. Neither the spectrum in Figure 8 nor that in Figure 9 used the TOSS pulse sequence, so spinning sidebands are prominent in both.
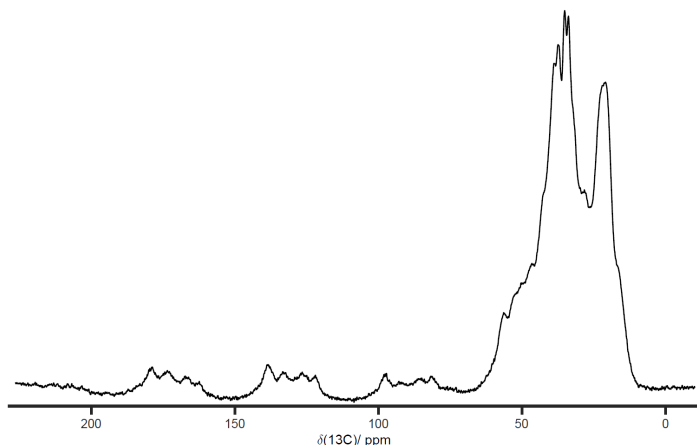
Figure 8. The 75 MHz solid state ^{13}C spectrum recorded at Lund University of sample 1735, provided by Priwan Lohitsean.
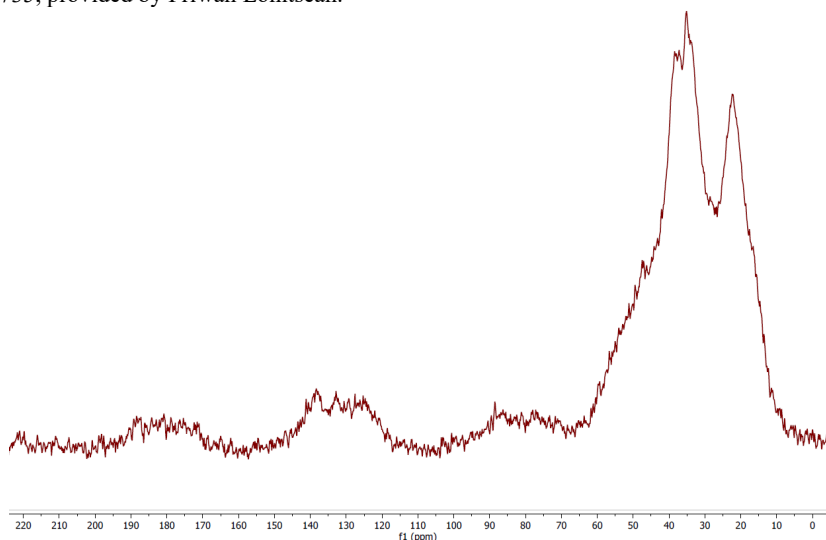

Figure 9. The 75 MHz solid state ^{13}C spectrum recorded at Northwestern University of sample 1735, provided by Priwan Lohitsean.
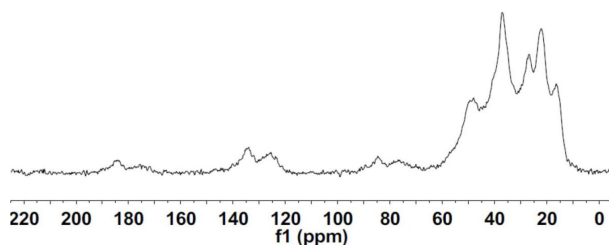
Figure 10. The 75 MHz solid state ^{13}C spectrum recorded at Northwestern University of sample 1418 from Kuala Tungkal, Sumatra, Indonesia (Figure 2 in Lambert et al. 2013).

Proton spectra have been found to be highly diagnostic of Group B (Lambert et al. 2013), so the ^{1}H spectrum of sample 1735 was recorded at Trinity University and is presented in Figure 11. This spectrum indeed is typical for Group B ambers, except for the peak at δ 1.6, part of which is attributed to water (and identifiable by the addition of more water). The water peak also is present in the spectrum of Group A sample 1768 in Figure 3. In both cases, the water peak is evident primarily because of the weakness of the spectra. Moreover, the spectrum in Figure 11 cannot be of a Group A amber because it lacks the sharp, middle peak of the three diagnostic peaks as seen in Figures 3, 5, and 7. Figure 12 illustrates a typical Group B ^{1}H spectrum lacking an enhanced water peak, in this case of sample 1418 from Kuala Tungkal, Sumatra, Indonesia, provided by Mohammad Iskandar bin Marzuki (Lambert et al. 2013).
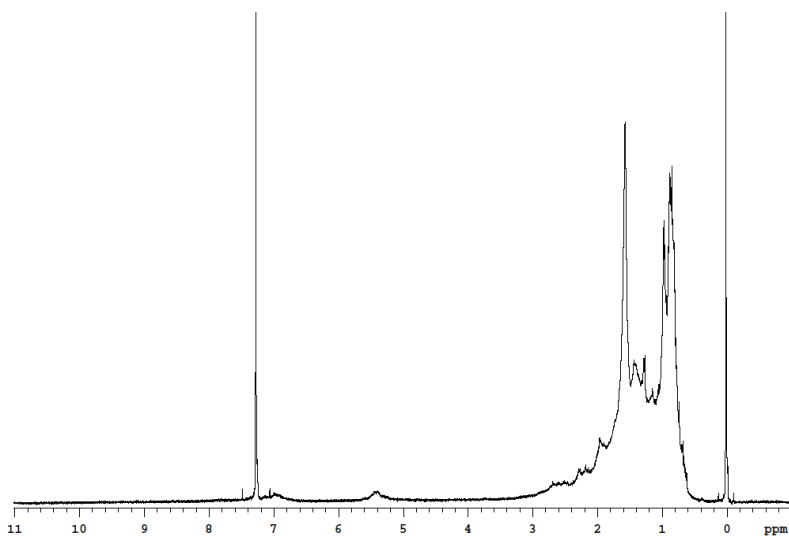

Figure 11. The 500 MHz ^{1}H spectrum recorded at Trinity University of sample 1735 in CDCl$_3$, provided by Priwan Lohitsean. Some of the intensity of the peak at δ 1.6 is from water impurity.
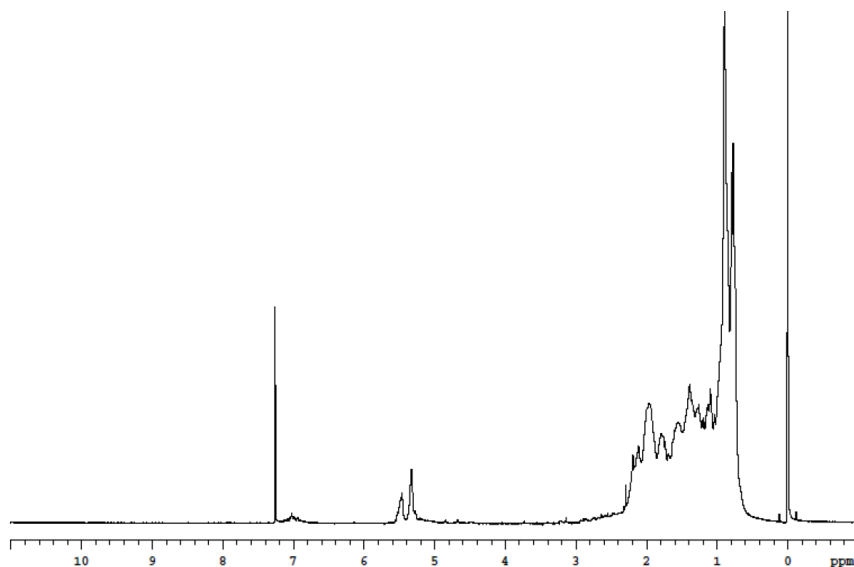
Figure 12. The 500 MHz ^{1}H spectrum recorded at Trinity University of sample 1418 from Kuala Tungkal, Sumatra, Indonesia, in CDCl₃ (Figure 2 in Lambert et al. 2013).

The ^{13}C and ^{1}H spectra of sample 1735 are characteristic of a Group B amber and definitely rule out classification as Group A. In the process of examining all our previously analyzed Burmese samples, the solid state ^{13}C spectrum sample 154 (Figure 13, labeled simply as Burmese amber without further geographical designation and provided by George O. Poinar Jr.) appeared to be different from the others. Comparison to Figures 8-10 indicates a previously unappreciated closer resemblance to the spectra of Group B ambers than to those of Group A ambers, as in Figures 2, 4, and 6. For confirmation, we recorded the ^{1}H spectrum, as seen in Figure 14, which is a perfect match to other definite Group B ambers, as in Figure 12. Thus, we report herein two Group B ambers from different Burmese sources.
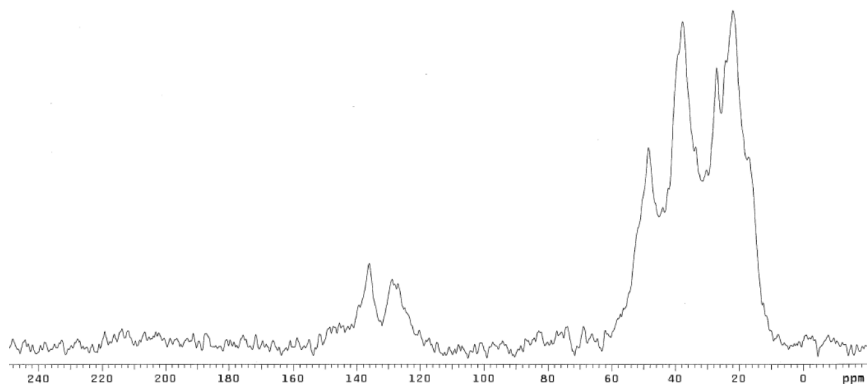
Figure 13. The 75 MHz solid state ^{13}C spectrum recorded at Northwestern University of sample 154 from Burma, provided by George O. Poinar Jr.
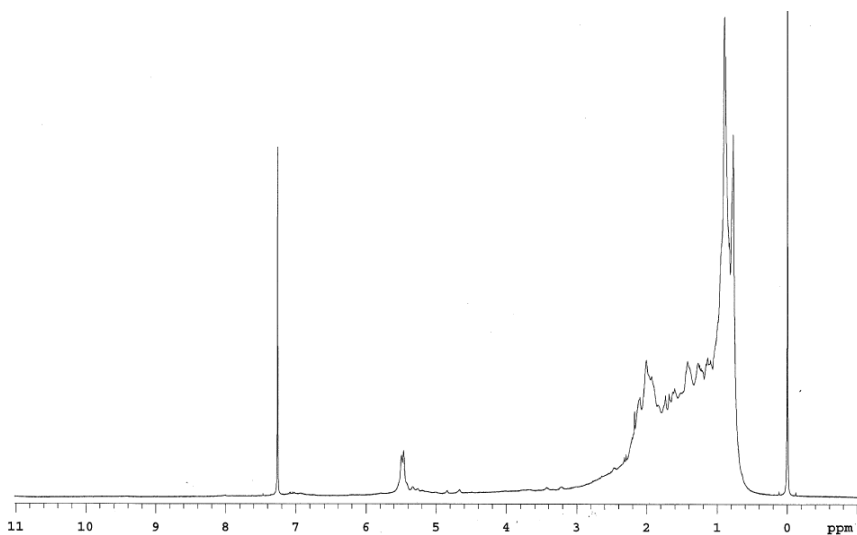

Figure 14. The 500 MHz ^{1}H spectrum recorded at Trinity University in CDCl$_3$ of sample 154, provided by George O. Poinar Jr.

Conclusions

All previous ambers characterized by NMR spectroscopy in mainland Southeast Asia have been from Group A, which came from conifer sources such as the Araucariaceae. We now have discovered two Burmese amber samples that clearly are from Group B, which came from angiosperm sources such as the Dipterocarpaceae. Modern dipterocarps from such genera as *Shorea*, *Hopea*, *Dipterocarpus*, and *Vatica* are common in Southeast Asia. Thus, Burma joins Japan, India, Australia, and the United States (Lambert et al. 1990, 1993, 2015)

in producing both Group A and Group B ambers in relatively close vicinities. In contrast, all analyzed amber from Indonesia has proved to be only Group B (Lambert et al. 2013). Zherikhin and Ross (2000) noted that several researchers have documented apparent chemical variation of Burmese amber but do not report chemical details.

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