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Supporting Online Material for
Identification of Carboniferous (320 Million Years Old) Class Ic Amber

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Supporting Online Material

Identification of Carboniferous (320 Ma) Class Ic Amber

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Materials and Methods

Macroscopic blebs of amber, measuring approximately 5 mm across, were handpicked using stainless steel picks from a mine-fresh Illinois, USA, coal from the Lower Desmoinsian Series, Tradewater formation that has been stratigraphically dated to ~320 Ma. Collection site will remain anonymous at the request of the owners and to preserve the site for future research. Reference samples have been archived with the American Museum of Natural History, New York. Fractured amber pieces were examined using a stereoscopic microscope to ensure purity (defined here as the absence of extraneous material such as adhering coal). Pure samples were analyzed using Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS). Py-GC-MS analyses of the amber were performed as previously described (*SI*), using an Agilent 6890 Gas Chromatograph with an Agilent 5973 Mass Selective Detector. Samples of ~200-400 µg were pyrolyzed at $T_{Py}=480^{\circ}\text{C}$ or $T_{Py} = 300^{\circ}\text{C}$ to differentiate occluded volatile terpenes and macromolecular materials. Methylation of acidic functional groups achieved *in situ* by co-pyrolysis with tetramethylammonium hydroxide (TMAH). The resultant pyrolysate

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was automatically transferred to the column via a split/splitless injector with a split ratio of 25:1. The carrier gas was He held at 1 ml/minute. Separation was achieved using a 60 m ZB-5ms capillary column, with a temperature programming as follows: $T_{\text{initial}}=40^{\circ}\text{C}$ (4 min); ramp = $4^{\circ}\text{C}/\text{min}$ to $T_{\text{final}}=280^{\circ}\text{C}$. Final temperature was then held isothermally for at least 16 minutes. Individual analytes are identified based on comparison of MS data with literature and library data and in some cases, by comparison of MS results and chromatographic behavior with those of known references. Co-pyrolysis studies were performed as above excepting the omission of analyses with $T_{\text{py}} = 300^{\circ}\text{C}$.

References and notes

S1. K.B. Anderson, *Geochem Trans* **7:2** doi:10.1186/1467-4866- 7-2 (2006).