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A simple and convenient determination of perylene preserved in the Late Neogene wood from northeastern Tennessee using fluorescence spectroscopy

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ABSTRACT

A new and convenient fluorescence spectroscopic method has been developed for the separation and determination of perylene in fossil wood recovered from a late Miocene to early Pliocene Gray Fossil Site in Tennessee, USA. This is the first report where perylene was separated from fossil samples from this newly discovered fossil site. The chemical structure of perylene was elucidated and confirmed by UV–visible and ^{1}H NMR spectroscopies, and ESI-TOF high accuracy mass spectrometry. The fluorescence of perylene was used to measure the contents of perylene in the fossil conifer and oak wood, which were found to be 5.34 and 0.78 μg/g, respectively. The results suggested that the amount of perylene in the fossil conifer wood was 7-fold higher than that in the oak wood. The method, which uses highly sensitive fluorescence spectroscopy, should provide a new approach for the analysis of other fossil and geochemical samples containing perylene.

1. Introduction

A recently discovered fossil site from northeastern Tennessee, now known as the Gray Fossil Site, represents an unusual late Tertiary biota (Wallace and Wang, 2004). This is a highly significant discovery because this is the only Neogene site in eastern North America where a diverse array of plant and animal fossils are well preserved together, and the geological age is constrained by the fossil mammals from the same layer as the fossil plants (Parmalee et al., 2002; Shunk et al., 2006). The fossil mammals have provided evidence for the existence of a late Miocene to early Pliocene terrestrial biota from a forested ecosystem and the contiguous connections between the fauna of eastern North America and eastern Asia (Wallace and Wang, 2004). Moreover, many plant fossils, such as leaves and acorns of *Quercus* (oaks) and nuts of *Carya* (hickories) were found relatively abundant at the site. Fossil pine needles and conifer wood also occur occasionally at the site. The fossil plants are mostly very well preserved, making further anatomical and molecular paleobotanical analyses possible. Recent studies of molecular paleobotany have shown promising results in finding biomarkers and other chemical compositions in plant fossils (e.g. Van Bergen et al., 1999; Otto et al., 2005; Marynowski et al., 2007).

Perylene or perilene is a polycyclic aromatic hydrocarbon with the chemical formula $C_{20}H_{12}$ (Fig. 1) and has frequently been reported from fossil wood (Otto et al., 2005; Bechtel et al., 2007). It is deposited widely in marine and fresh water sediments and soils. There are three possible processes to produce perylene: (1) early diagenesis from well defined biogenic precursor molecules, (2) catagenetic alterations of biogenic precursor materials, and (3) high temperature fragmentation and recombination of organic matter (Silliman et al., 2001). Most perylene is from high

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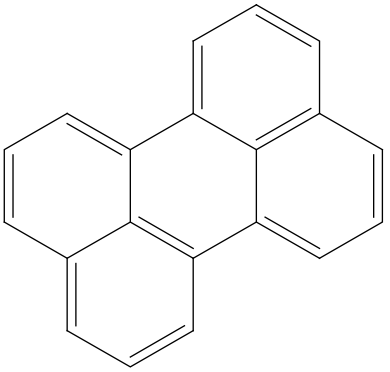

Fig. 1. Perylene chemical structure.

temperature anthropogenic processes (Silliman et al., 1998). Perylene is able to resist alteration and degradation in sediments. This makes perylene a useful chemical biomarker for fossil wood and sediments (Otto et al., 2005; Bechtel et al., 2007).

Perylene is a fluorescent molecule with a blue fluorescence and an absorption maximum at 434 nm. Perylene has a molar extinction of 38,500 M^{-1} cm^{-1} in cyclohexane at 434.75 nm. When a wavelength of 410 nm was used for the excitation of perylene in cyclohexane, the maximum emissions appeared around 450 nm, which was unique for perylene (Du et al., 1998). Other naturally occurring polycyclic hydrocarbons (Silliman et al., 2001), such as pyrene, benzo[*a*]pyrene, fluoranthene and phenanthrene had excitation maxima at 345, 390, 360 and 322 nm, respectively, with emission at 395, 408, 500 and 376 nm, respectively (Kershaw, 1996; Beltran et al., 1998; Du et al., 1998; Jiang et al., 2001). As a result, perylene is used as a fluorescent probe for cell biology and cytochemistry. This paper describes a novel and convenient method to separate perylene from fossil wood and a sensitive assay using unique perylene fluorescence emission around 450 nm, to quantify the contents of perylene in fossil conifer and oak wood.

2. Samples and methods

2.1. Reagents and general methods

All chemicals were purchased from Sigma–Aldrich and other chemical sources and used without further purification. Standard perylene was obtained from *Alfa Aesar* with 98% purity. A sonicator (FS9) was from Fisher Scientific. Flash chromatography was performed with silica (Merck, Grade 9385, 230–400 mesh) and monitored by thin layer chromatography (TLC) with silica plates (Merck, Kieselgel 60 F254). The melting points were determined using MEL-TEMP. The ^{1}H NMR spectra were obtained using a 400 MHz Varian instrument using deuterochloroform ($CDCl_3$). The chemical shifts of protons were given in ppm with TMS as an internal standard. Fluorescence spectroscopic measurements were performed on a FluoroMax-3 from HORIBA JOBIN YNON. A 4 ml quartz fluorescence cuvette was used, slit widths of the fluorometer were set to 2 for both windows, and the excitation wavelength was 410 nm. The final volume of solutions for all the measurements was 2 ml. UV–vis absorption spectra were recorded on a Shimadzu UV-1700 UV–visible spectrophotometer. All the measurements were carried out at 298 K. Exact mass of perylene was recorded on electrospray ionization-time of flight accurate mass analysis (ESI-TOF-acc) at the Scripps Mass Spectrometry Center.

2.2. Fossil sample collection

Dry fossil wood samples were collected from finely laminated black clays outcropping at the Gray Fossil Site, dated as the latest Miocene to earliest Pliocene (7–4.5 million years BP). Dating was based on the co-occurrence of two mammal fossils, the rhino (*Teleoceras* sp.) and the short faced bear (*Plionarctos* sp.) (Wallace and Wang, 2004; Shunk et al., 2006). A preliminary study of the fossil wood showed that two groups, pinaceous (*Pinus* sp.) and oaks (*Quercus* sp.), were frequently represented (M. Zavada and G. Feathers, personal communication).

2.3. Separation and identification of perylene from fossil conifer wood

Fossil conifer wood (197 g) was ground to a fine powder and sonicated three times for 10 min each with 200 ml of a mixed solvent (dichloromethane/methanol, 1/1, v/v). The solvent extracts were filtered, combined and concentrated using a rotary evaporator. The residue was purified with column chromatography for three times. The columns were prepared by suspending silica gel (37 g) in hexane. The columns were eluted with hexane, then dichloromethane, and cyclohexane. The sequence of hexane followed by dichloromethane yielded perylene, which was recrystallized further from acetic acid (0.4 ml) to floppy and golden perylene crystal flakes (0.1 mg). m.p. 275–277 °C [lit. (Porwoll, 2007), 276–279 °C]; R_f = 0.15 (cyclohexane); ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.475 (*t*, *J* = 7.6 Hz, H2, 4 H), 7.684 (*d*, *J* = 8.4 Hz, H3, 4 H), 8.197 (*d*, *J* = 7.6 Hz, H1, 4 H) [lit. (Abraham et al., 2000), 7.466 (H2), 7.656 (H3), 8.196 (H1)]. UV–visible λ_{max} (cm^{-1}): 408 and 436 [lit. (Du et al., 1998), 407 and 436]. ESI-TOF-acc (M + H) calculated for $C_{12}H_{17}O_4$, 225.1121. Observed: 225.1124.

2.4. Standard curve and measurement of perylene concentrations

A titration was carried out in cyclohexane (2 ml) by measuring intensities of perylene fluorescence at 437 nm with increasing final concentrations of perylene from 0, 0.0147, 0.0295, 0.059, 0.118, 0.223, 0.354 to 0.472 μM (Fig. 2). A plot of the intensities of fluorescence against concentrations of perylene generated a straight line, which was a linear range of a standard curve for perylene concentration (Fig. 3).

To determine the perylene concentrations, the intensity of perylene fluorescence of a fossil sample was located on the *Y*-axis (Fig. 3). A line parallel to the *X*-axis was drawn through the spot on the *Y*-axis to intersect the standard curve, and a vertical line was then drawn through the standard curve to intersect the *X*-axis. The corresponding value on the *X*-axis was the concentration of perylene in a fossil sample.

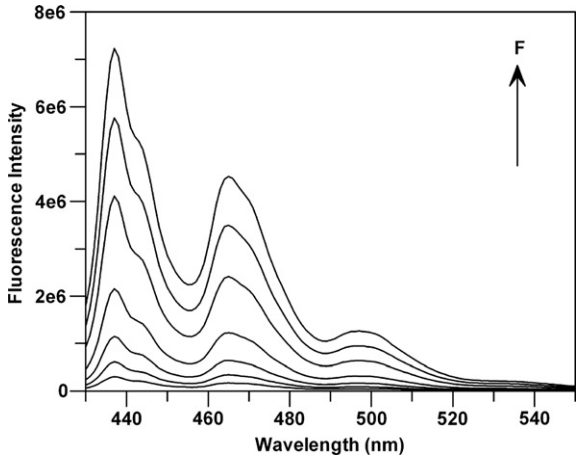

Fig. 2. Increase of perylene fluorescence intensities with increase of perylene concentrations from 0, 0.0147, 0.0295, 0.059, 0.118, 0.223, 0.354 to 0.472 μM in cyclohexane.

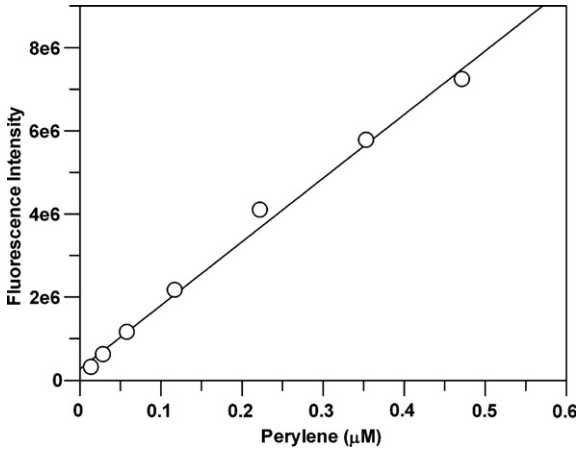

Fig. 3. Plot of perylene fluorescence intensity against perylene concentration, which was a linear range of a standard curve.

2.5. Separation and measurement of perylene in fossil conifer wood

Conifer fossil wood (1 g) was ground to fine powder and extracted twice each with 20 ml of dichloromethane/methanol (1/1, v/v). The solvent extracts were filtered, combined and concentrated, and the residue was purified with column chromatography using silica gel (5 g). The column was prepared with hexane but eluted with cyclohexane. The fractions from the fossil conifer wood were checked by UV at 254 nm for the presence of perylene, combined and evaporated to a residue (4 ml), which was used for the determination of perylene concentrations and photographs. The residual solution (0.16 ml) was diluted with cyclohexane to 2 ml solution, and then measured for perylene using a fluorometer. Perylene in fossil oak wood was separated and analyzed using a similar procedure with the additional analysis of fractions collected from column chromatography.

3. Results and discussion

3.1. Separation and determination of perylene in fossil conifer and oak wood

The fluorescence spectra of perylene from both samples (Fig. 4) were identical to standard samples (Fig. 5). This confirms that perylene concentrations can be effectively measured using fluorescence intensity. Although the presence of perylene in solution was visible to the naked eye (Fig. 6), the concentrations of perylene in fossil conifer and oak wood were quantified using the standard curve (Fig. 3). The contents of perylene in fossil conifer and oak wood were found to be 5.34 and 0.78 μg/g, respectively. These results showed for the first time that the concentration of perylene in fossil conifer wood was 7-fold higher than that of perylene in the fossil oak wood.

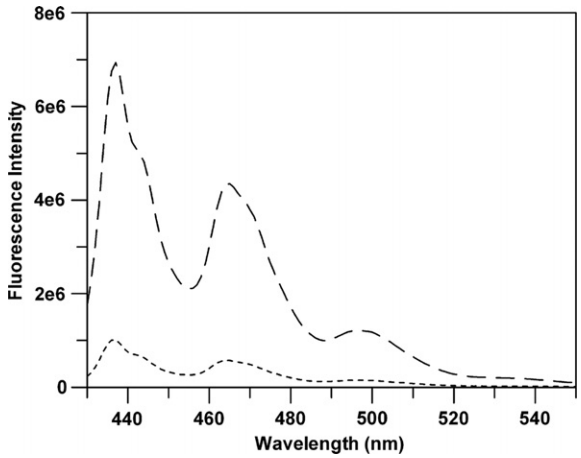

Fig. 4. Fluorescence spectra of perylene purified from fossil conifer (0.42 μM, dash) and oak (0.061 μM, dot) wood.

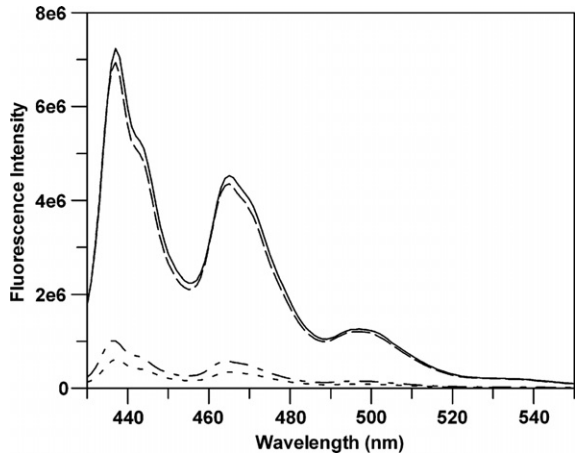

Fig. 5. Match up of fluorescence spectra of perylene purified from fossil conifer (0.42 μM, dash) and oak (0.061 μM, dot) wood with standard samples (0.472 μM, solid line; 0.0295 μM, dash–dot).

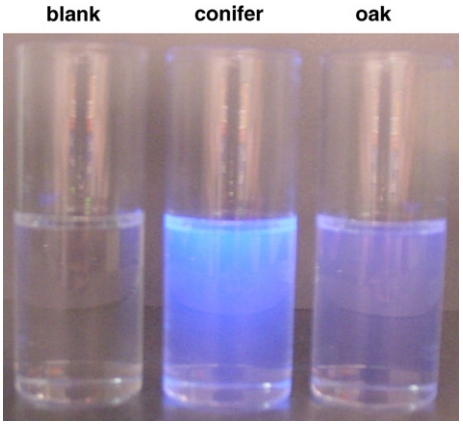

Fig. 6. Perylene fluorescence of samples (4 ml each), blank (left), fossil conifer wood extract (middle, 5.25 μM), fossil oak wood extract (right, 0.76 μM) in cyclohexane, respectively.

4. Summary and conclusions

The Gray Fossil Site in Tennessee is an important site for well preserved plant and animal fossils. In this paper, we isolated perylene from both the fossil conifer and oak wood by grinding the wood samples, extraction of the powder, and purification of the extract using column chromatography. Perylene structure was identified using UV–visible, fluorescence and NMR spectroscopies and ESI-TOF-acc. Using fluorescence measurements we determined that the content of perylene in conifer wood fossils was 7-fold higher than in oak wood fossils, 5.34 and 0.78 μg/g, respectively. The fluorescence assay was shown to be a sensitive and simple method for the quantification of perylene in fossil wood. This method could also be applied to the determination of perylene in other fossil wood and geochemical matrices (e.g., soils).

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