



Exact and Quantitative Analysis of Deuterated Pyrene. A New Method for the Rapid, Convenient Hydrogen-Deuterium Exchange of Polycyclic Aromatic Hydrocarbons.

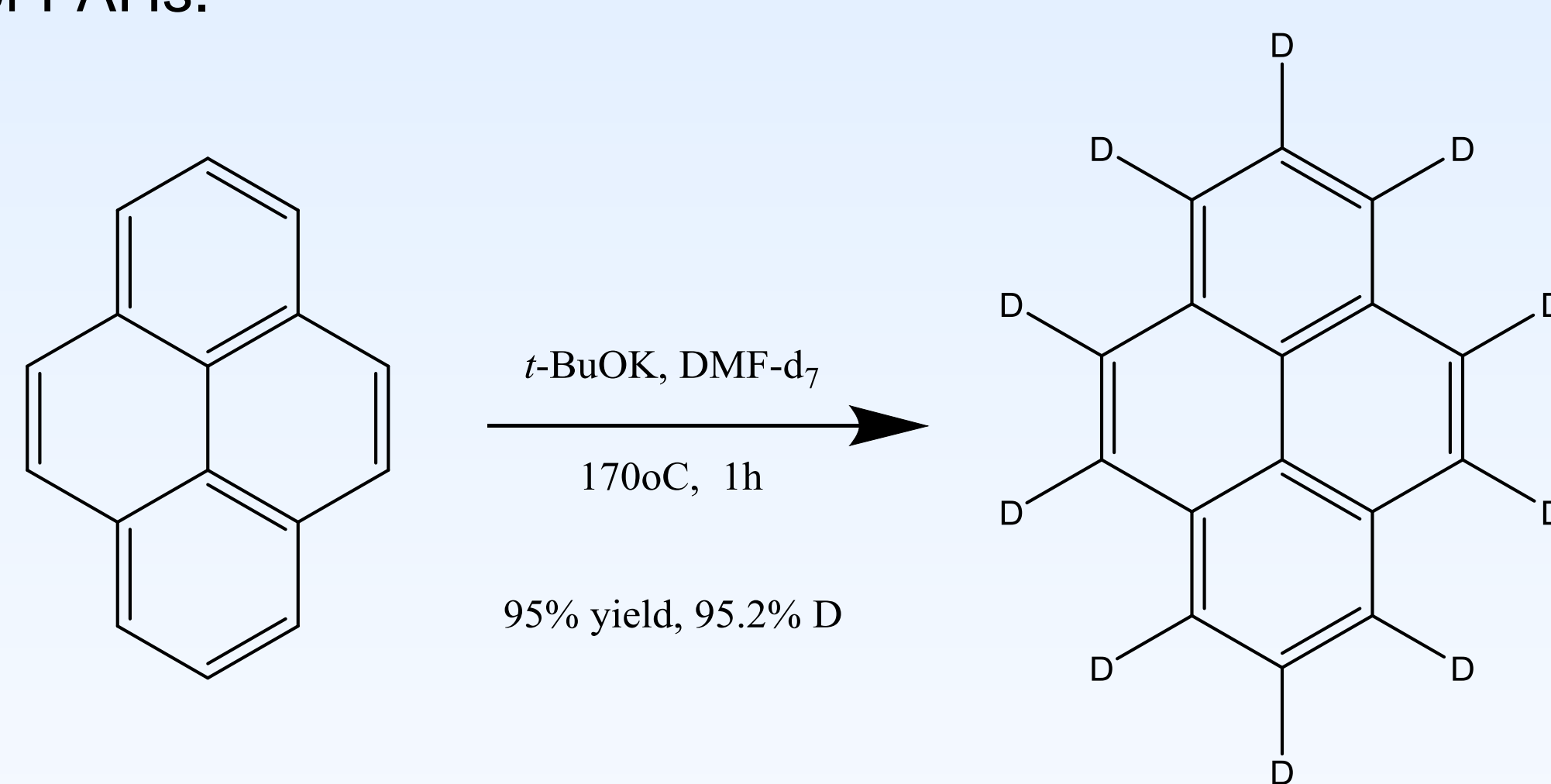
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Overview

Deuterated polycyclic aromatic hydrocarbons (PAHs) are widely used as analytical standards for monitoring environmental pollutants; however, only the most common PAHs are commercially available fully deuterated. We have therefore developed a general method for the hydrogen deuterium exchange in PAHs. In this HDX-MS study, the deuterium level is determined by the mass shift using direct analysis in real time (DART) with Cerno Biosciences MassWorks software. The determination of deuterium levels of the mixture is complicated, as the native and multiple labeled forms elute together at the same retention time in liquid chromatography (LC). This work will report a novel method to calculate the relative amounts of all relevant components to quantitatively assess the contributions of individual deuterium labeled forms to the mixture MS signal and to determine the average number of deuterium atoms in the mixture.

Introduction

Over the years there have been many hydrogen/deuterium (H/D) exchange methods of polycyclic aromatic hydrocarbons studied.¹ One of the major drawback of all the current methods is that specialized apparatus, high temperatures, and/or long reaction times are required. Here at Boston College, Professor Scott's group has developed a rapid, low-cost method that uses commercially available DMF-d₇ as the deuterium source and potassium *tert*-butoxide as a basic catalyst,² in combination with microwave irradiation to promote the hydrogen deuterium exchange of PAHs.



Conversion of pyrene to pyrene-d₁₀ with >98% deuterium incorporation in just 1h of microwave irradiation in a solution of *t*-BuOK/DMF-d₇.

Due to the fact that insoluble PAHs as well as other classes of insoluble samples are traditionally difficult, if not impossible to analyze using conventional liquid-based ionization methods, we have decided to use Direct Analysis in Real Time (DART). DART is capable of analyzing samples with no need for special sample preparation or instrument setup.³ The high-quality data thus obtained with a DART ionization source coupled to a TOFMS along with Cerno's MassWorks software has allowed us to monitor and determine the deuterium incorporation in a matter of minutes.

Methods

All chemicals were purchased and used without further purification unless otherwise noted. Deuterated materials, *N,N*-dimethylformamide-d₇ (99.5% D) and pyrene-d₁₀ (98% D), were purchased from Cambridge Isotope Laboratories. Potassium *tert*-butoxide was purchased from Aldrich and stored sealed under nitrogen in a desiccator. Exposure to moisture resulted in diminished deuterium incorporation. Microwave irradiation was performed with a CEM Discover LabMate reactor with IntelliVent pressure control system; temperatures of the reaction mixtures were monitored by a vertically focused IR temperature sensor and not by an internal temperature probe.

All experiments were carried out on a JEOL AccuTOF instrument (JEOL USA, Peabody, MA), equipped with a DART ion source (IonSense, Inc., Danvers, MA) in positive ion mode. Ultra high purity helium was used at a flow rate of 4L/min to generate the metastables. The following standard DART source settings were used for all experiment, with the exception of heater temperature, which was set from 220-500°C: needle voltage 3500V, discharge electrode 150V and grid electrode 250V. Data were collected over a scan range of *m/z* 100-800.

All spectra were acquired simply by inserting the closed tip of a melting point tube into the sample vial, making sure that good contact was made between the melting point tip and the sample. The tip of melting point was then held in the ion beam of the DART source for a period of 3-5sec.

Before and/or after sample acquisition, a solution of polyethylene glycol (PEG) was used to calibrate the spectra. The calibration solution was prepared by dissolving 50μl of PEG(300 and 600) in 100ml of 50:50 methanol:dichloromethane.

After an MS calibration to obtain a mathematically defined and symmetric MS peak shape, a least squares fit between the mixture MS profile and those calculated for the various components to arrive at the relative amounts of the various isotopologs that differ only in the number of deuterium atoms.

Pyrene-1,2,3,4,5,6,7,8,9,10-d₁₀

To a flame-dried, nitrogen purged 10ml microwave vessel equipped with a magnetic stir bar were added 50mg (0.248 mmol) of pyrene and 1ml of DMF-d₇. The mixture was stirred and sublimed potassium *tert*-butoxide was added (0.555g, 4.95mmol). The microwave vessel was capped and placed in a microwave reactor for 1h at 170°C. The mixture was cooled to room temperature and immediately flushed through a short pad of silica gel with dichloromethane as the eluent. Evaporation of the solvent provided 49.9mg (95%) of deuterated pyrene as a tan solid.

Fluoranthene-1,2,3,4,5,6,7,8,9,10-d₁₀

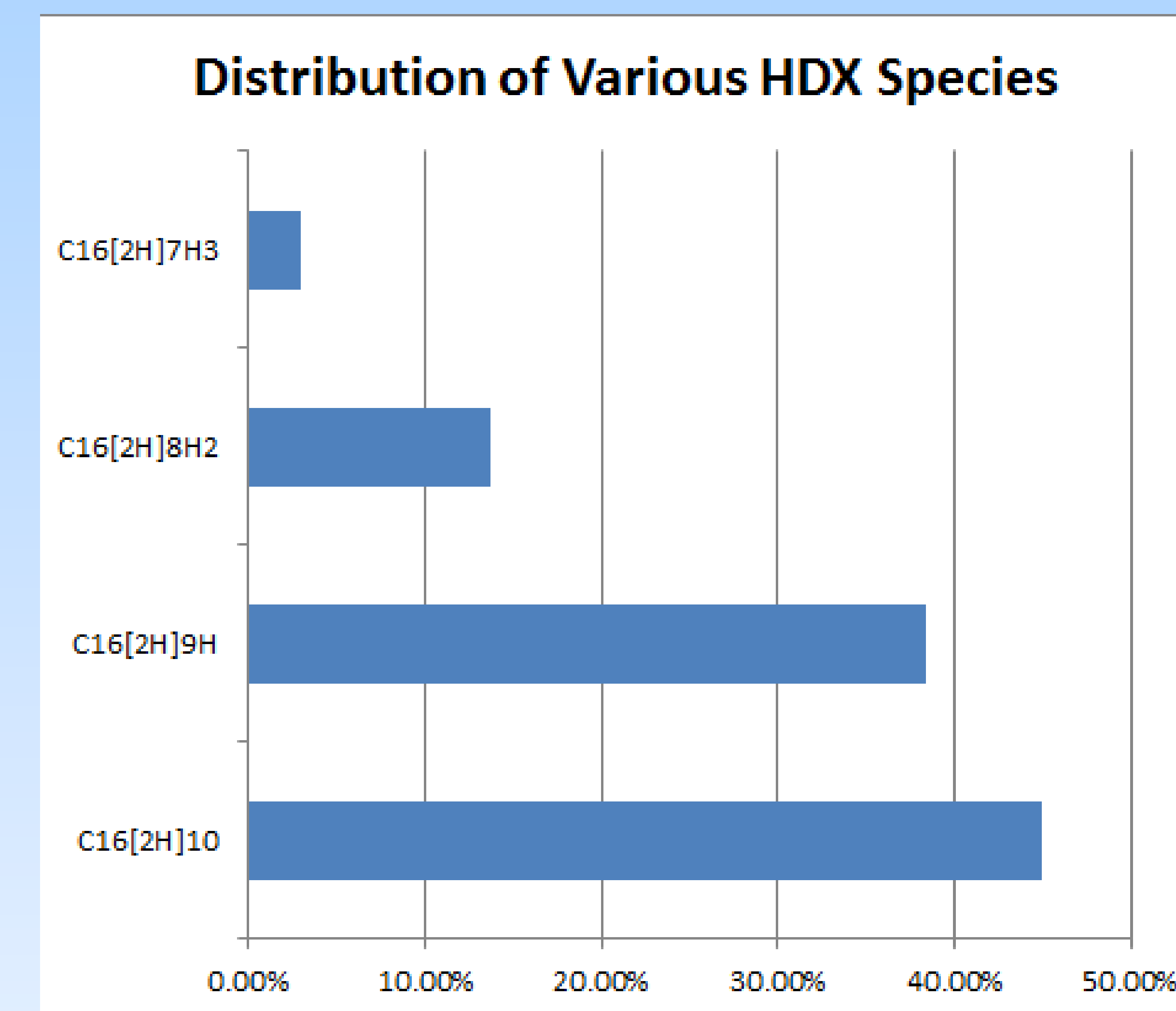
The same general procedure above was used with fluoranthene (50mg, 0.248mmol), 1ml dimethylformamide-d₇, and sublimed potassium *tert*-butoxide (0.555g, 4.95mmol) to afford 39mg (75%) of deuterated fluoranthene as a tan solid.

Corannulene-1,2,3,4,5,6,7,8,9,10-d₁₀

The same general procedure above was used with 20mg (0.08mmol) of corannulene, 0.4ml of dimethylformamide-d₇, and sublimed potassium *tert*-butoxide (0.179g, 1.6mmol) to afford 16.7mg (80%) of deuterated corannulene as a tan solid

Results

Exact quantitation of the deuterium level from the HDX-MS data is typically a challenging task due to both the overlapping ion signals and the fact that the MS peak shape is not defined or even known. This new approach for exact MS quantitation has successfully calculated the relative amounts of two components with overlapping MS signals with TOF MS data. With the enhanced algorithmic and software capability, the same approach has now been applied to analyze MS data with multiple deuterium-labeled isotopologs.



Conclusions

Our initial work using an example of a singly charged ion for corannulene at *m/z* 202 showed dominant HDX forms with 10 deuterium atoms and smaller amounts with 9, 8 and 7 deuterium atoms in the mixture. This information typically could not be obtained from any calculation without mass spectral deconvolution. For example, a mass spectral shift of three atomic mass units may be due to a single component with three deuterium atoms but may also be due to a component containing a mass spectral peak arising from the ¹³C isotope peak of a compound labeled with two deuterium atoms. With the relative amount of individual isotopologs available, the average number of deuterium atoms in the HDX mixture can be accurately calculated. The advantages of this new approach will be further illustrated by comparing the average mass shift, the average number of deuterium atoms involved, and the above relative amounts.

References

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