Origin of Polycyclic Aromatic Hydrocarbons (PAHs) from the Combustion of Biomass using $^{13}$C-Labeling and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS)

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How Does He Do It?
How Does He Do It?
Formation of PAHs in Complex Systems

- **Goal:** Uncover the precursors to polycyclic aromatic hydrocarbons (PAHs) in a burning cigarette

- **Background:**
  - PAHs thought to form from hydrocarbon component of tobacco (paraffins, steroids, terpenes, esters fatty acids)
  - Pyrolysis of hexane extract of tobacco produces 60% benzo[a] pyrene found in pyrolysate and the pyrolysate posses tumorigenic activity *(Beitr. Tobkforsch 1973, 7, 165; Cancer 1958, 12, 1140)*
  - PAH yields: **time**, temperature, and concentration
  - What are the reaction conditions in a burning cigarette?
**Classic View of a Burning Cigarette**

- **Combustion zone**
  - Temperature: 700 - 900 °C
  - Oxygen consumed by combustion of carbonized tobacco
  - CO₂, CO, H₂O and heat generated

- **Pyrolysis/Distillation zone**
  - Temperature: 200 - 600 °C
  - Oxygen concentration: 0 - 4%
  - Volatile organics produced

- **Puff**
  - Air velocities ≤400 cm s⁻¹
  - Heating rates: ≤500 °C s⁻¹
  - Residence time of volatiles
    - Combustion zone: <1 ms
    - Pyrolysis zone: <5 ms

- **Smoldering**
  - Convection air flow
  - Heating rates: 2-10 °C s⁻¹
  - Residence time of volatiles
    - Hundreds of ms
    - 1-10X more PAHs in SS than MS
Pyrolysis of Hydrocarbons Investigated

Cholesterol \( R = \text{HO—} \)
Cholesteryl stearate \( R = \text{CH}_3(\text{CH}_2)_{16}\text{CO}_2— \)
Cholesteryl oleate \( R = \text{CH}_3(\text{CH}_2)_{7}\text{CH=CH(CH}_2)_{7}\text{CO}_2— \)
Cholesteryl linolenate \( R = \text{CH}_3(\text{CH}_2\text{CH=CH})_3(\text{CH}_2)_{7}\text{CO}_2— \)

Squalene (C\text{\textsubscript{30}})

Stigmasterol

\( \text{β−Carotene (C\text{\textsubscript{40}})} \)

Phytol (C\text{\textsubscript{20}})

Solanesol (C\text{\textsubscript{45}})

A New Analytical Approach Inspired by Colin for Investigation of PAHs

- **Problem:** The temperature and residence times of tobacco components are unknown during smoke formation. Thus, their potential for producing PAHs is unknown.

- **Solution:** Study reactions in a burning cigarette

- **Compound Specific Carbon Isotope Measurement**
  - Variation in the Stable Isotope ratios of Specific Aromatic and Aliphatic Hydrocarbons from Coal Conversion Processes *Analyst* 1998, 123, 1519
  - Use of $^{13}$C Labeled Compounds to Probe Coke Formation in FCC *Preprints - ACS, Division of Petroleum Chemistry* 1999, 44(4), 481
Product-Precursor Relations in Smoke

- **Hypothesis:**
  - GC-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) can be used to determine the precursor to polycyclic aromatic hydrocarbons (PAHs) found in mainstream smoke
  - GC-C-IRMS can bridge the gap between reactions in a burning cigarettes and pyrolysis or combustion reactions

- **Approach:**
  - Spike 2R4F cigarettes with $^{13}$C-labeled tobacco precursors (cholesteryl stearate-3,4-$^{13}$C$_2$) and isolate PAH rich fraction
  - Compare $^{13}$C content of phenanthrene, benz[a]anthracene, and benzo[a]pyrene (BaP) before and after spiking

- **Major challenges in experiments:**
  - Separation of PAHs from TPM (baseline resolution)
  - Sensitivity of GC-C-IRMS
\[ \delta^{13}C = \left( \frac{^{13}C/^{12}C}_{\text{sample}} \right) - \left( \frac{^{13}C/^{12}C}_{\text{reference}} \right) - 1 \times 10^3 \]

Oxidation Reactor
NiO / CuO / Pt / °C

injector
to FID or closed
X-piece backflush

GC Capillary
double T-piece
Reduction Reactor
Cu/600 °C

He vent
Water Separator
Liquid Nitrogen Trap for CO₂

m/z 44 \(^{12}C^{16}O_2\)
m/z 45 \(^{13}C^{16}O_2\)
m/z 46 \(^{12}C^{16}O^{17}O\)

\((^{13}C/^{12}C) 10^{-5}\) on 0.5 nmol C
Delta Scale

$^{13}$C atom %

$\delta^{13}$C vs. PDB in [$^0/_{oo}$]

OAK RIDGE NATIONAL LABORATORY
U. S. DEPARTMENT OF ENERGY

Meier-Augenstein, W.
J. Chromatogr., A 1999, 842, 351
GC-C-IRMS

Trace GC with FID
GC Combustion III
Finnigan MAT 252 IRMS
ThermoFinnigan Isodat
GC Combustion III Interface
GC-C-IRMS of PAH Standards

Phenanthrene
Benz[a]anthracene
Benz[a]pyrene
## Precision of GC-C-IRMS of PAHs

<table>
<thead>
<tr>
<th>Shots</th>
<th>Phenanthrene ng</th>
<th>$\delta^{13}$C</th>
<th>Benz[a]anthracene ng</th>
<th>$\delta^{13}$C</th>
<th>Benzo[a]pyrene ng</th>
<th>$\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
<td>-25.56 ± 0.13</td>
<td>100</td>
<td>-26.33 ± 0.34</td>
<td>200</td>
<td>-24.34 ± 0.45</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>-26.05 ± 0.32</td>
<td>10</td>
<td>-26.14 ± 0.31</td>
<td>20</td>
<td>-24.82 ± 0.78</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>-25.90 ± 0.34</td>
<td>5</td>
<td>-26.22 ± 0.47</td>
<td>10</td>
<td>-24.0 ± 1.2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-25.69 ± 0.98</td>
<td>2</td>
<td>-26.3 ± 1.2</td>
<td>4</td>
<td>-24.3 ± 1.0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-25.5 ± 1.2</td>
<td>1</td>
<td>-25.90 ± 0.82</td>
<td>2</td>
<td>-24.2 ± 2.5</td>
</tr>
</tbody>
</table>

$\delta^{13}$C = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{reference}}} - 1 \right] \times 1000$

- Keep sample size >5 ng (ca. 0.4 nmol C) to obtain $\delta^{13}$C = ±1‰
Properties of 2R4F Cigarettes

- Second run of 1R4F reference cigarettes (Low Tar):
  - Contains 1.055 g/cigarette
    - Flue-cured 32.51%
    - Burley 19.94%
    - Oriental 11.08%
    - Maryland 1.24%
    - Reconstituted 27.13%
    - Glycerin 2.8%
    - Isosweet (sugar) 5.3%
  - TPM 11.7 mg/cig, FTC Tar 9.7 mg/cig, Nicotine 0.85 mg/cig
  - PAH yields in mainstream smoke
    - Phenanthrene: 125 ng/cigarette
    - Benz[a]anthracene: 14.5 ng/cigarette
    - Benzo[a]pyrene: 7.0 ng/cigarette
Dusted off Old Smoking Machine

Mainstream smoke generated FTC conditions:
- Puff duration 2 s
- Puff volume 35 mL
- Puff frequency once per min
- Cigarettes 75 F, 60% relative humidity for 48h
New Method Separation of PAHs from TPM

- Extract TPM from Cambridge pads with acetone
- Liquid-liquid extraction:
  - benzene:methanol:ether (2:1:2) vs water (3x)
- Silica Sep-Pak eluting with hexanes
  - TPM in THF precipitated into hexanes (>95% BaP)
- NH₂ Sep-Pak eluting with hexanes
  • Dumont et al. *J. Chromatogr. Sci.* 1993, 31, 371 (>95% BaP)
- Semi-Prep HPLC - Nova-Pak Silica (7.8 x 300 mm)
  - Elute at 4 mL/min hexanes with UV detection (280 nm) and strip with ethyl acetate - Take one fraction with all PAHs
- Semi-Prep HPLC - PAC and Ring-Sep (4.6 x 250 mm)
  - Elute at 1.5 mL/min 99% hexanes:1% CH₂Cl₂ with UV detection. Strip with 90% CH₂Cl₂:10% hexanes and 90% CH₂Cl₂ :10% isopropanol and return to 99% hexanes:1% CH₂Cl₂
  - Collect two fractions
PAHs on PAC-Ring Sep Column

PAHs

TPM

F#1

F#2
GC-C-IRMS (FID) Fraction #1

Phen

Retention time
GC-C-IRMS Fraction #2

BaA

BaP
Isotope Ratio for the PAHs in Smoke

<table>
<thead>
<tr>
<th>PAH</th>
<th>Run 1 (‰)</th>
<th>Run 2 (‰)</th>
<th>Run 3 (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phen</td>
<td>-29.78 ± 0.11</td>
<td>-28.63 ± 0.10</td>
<td>-28.29 ± 0.44</td>
</tr>
<tr>
<td>BaA</td>
<td>-31.63 ± 0.66</td>
<td>-27.81 ± 0.22</td>
<td>-27.36 ± 0.51</td>
</tr>
<tr>
<td>BaP</td>
<td>-4.9 ± 6.9</td>
<td>ND</td>
<td>-25.70 ± 0.27</td>
</tr>
</tbody>
</table>

- PAHs from sixty 2R4F cigarettes smoked under standard conditions
- Isotope ratio of PAHs were similar for the different runs made over 18 months apart.
- BaP in line with expectation
Cigarettes Spiked with PAH Precursor

- Cigarette spiked with cholesteryl stearate-3,4-\(^{13}\)C\(_2\)
  - Average concentration of sterols in tobacco 0.26 wt\% (2 studies)
  - Most steroids in tobacco are esterified
  - 20\% of cholesterol-4-\(^{14}\)C transferred to mainstream smoke
- Spiked with 10 wt\% of the total concentration of sterol (i.e., 0.026 wt\%) to minimize impact of additive
- Desired amount of \(^{13}\)C-labeled steroid was dissolved in hexanes and 20 \(\mu\)L injected via 50 \(\mu\)L syringe rotating syringe while slowly pulling out (Jenkins et al. *Tobacco Sci.* 1975, 19, 115)
- Smoked 60 cigarettes (6 per pad) under standard conditions and isolated PAHs as previously described
### $^{13}$C Content of PAHs in Cigarette Smoke

<table>
<thead>
<tr>
<th>Compound</th>
<th>2R4F (‰) unspiked</th>
<th>2R4F spiked with $^{13}$C-labeled steroid (‰)</th>
<th>0.026 wt%</th>
<th>Repeat</th>
<th>New 0.026 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>-28.90 ± 0.78</td>
<td>11.95 ± 0.68</td>
<td>2.51 ± 0.28</td>
<td>14.24 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>BaA</td>
<td>-28.9 ± 2.3</td>
<td>-9.9 ± 1.7</td>
<td>PC</td>
<td></td>
<td>28.0 ± 0.65</td>
</tr>
<tr>
<td>BaP</td>
<td>-25.70 ± 0.27</td>
<td>CP</td>
<td>CP</td>
<td></td>
<td>17.9 ± 1.5</td>
</tr>
</tbody>
</table>

PC = poor chromatography; CP = coeluting peak

- Great agreement with previous results for Phen and BaP isolated
- BaA has coeluting peaks and the number is suspect
- Phenanthrene was enriched when 0.0052 wt% cholesteryl stearate was used -23.88 ± 0.23
- **PAHs enriched in $^{13}$C indicates that cholesteryl stearate produces PAHs during smoking!**
Quantitation of $^{13}$C-Incorporation

- $\delta^{13}$C values can be converted into atomic fraction ($F$) and atomic percent ($AP = 100 \times F$)

\[
F = \frac{\frac{^{13}C}{^{13}C+^{12}C}}{\frac{^{12}C}{^{13}C+^{12}C}} = \frac{R_{sample}}{R_{sample} + 1} \quad R_{sample} = \left[ \frac{\delta^{13}C}{1000} + 1 \right] \times R_{VPDB}
\]

- Atomic percent excess ($APE$) = $AP_E - AP_B$ where $E$ is enriched sample and $B$ is background

- Mole percent excess ($MPE$) = $APE / (\text{number of labeled carbons/total number of carbons in PAH})$; for phenanthrene the denominator is $2/14$
### Role of Steroids in PAH Formation

<table>
<thead>
<tr>
<th></th>
<th>Phenanthrene</th>
<th>BaA</th>
<th>BaP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MPE (10 wt%)</strong></td>
<td>0.320%</td>
<td>0.214%</td>
<td>PC</td>
</tr>
<tr>
<td><strong>MPE (Repeat 10 wt%)</strong></td>
<td>0.238%</td>
<td>PC</td>
<td>PC</td>
</tr>
<tr>
<td><strong>MPE (10 wt% New)</strong></td>
<td>0.313%</td>
<td>0.540%</td>
<td>0.465%</td>
</tr>
<tr>
<td>Average yield (µg/g)</td>
<td>1.45 ± 0.2</td>
<td>-</td>
<td>0.13</td>
</tr>
<tr>
<td>Average % Total PAH</td>
<td>2.9 ± 0.5%</td>
<td>-</td>
<td>4.65%</td>
</tr>
</tbody>
</table>

- **Assumptions:**
  - PAH yields similar to that reported for 2R4F
  - Two $^{13}$C-labels are found in the PAHs
  - Cholesteryl stearate representative of all tobacco steroids
- Phenanthrene yield lower than that found from pyrolysis of stigmasterol at 600 °C and 1 s (56.6 µg/g)
- **Steroids play a small role in PAH formation in MS smoke**
Summary and Conclusions

- GC-C-IRMS can be used to measure isotopic content of specific smoke constituents and quantitatively determine precursor-product relationships under smoking conditions.
- Technique has good sensitivity and reproducibility, but it is limited by the availability of $^{13}$C-labeled reagents, complexity of the sample cleanup, and chromatography.
- Steroids are minor contributors (<5%) to the PAHs found in mainstream smoke.
- Technique can be used to investigate reaction pathways of tobacco components in a burning cigarette (Determine reaction intermediates by looking for the peaks with excess $^{13}$C).
- This technique can also be extended to oxygen, nitrogen isotopes (N-PACs), and deuterium.
CONGRATULATIONS COLIN!