Characterization of Nitrobenzanthracenes and Nitrodibenzanthracenes

BERIT IVERSEN, a LEIV K. SYDNES b and TYGE GREIBROKK a

a Department of Chemistry, University of Oslo, P.B. 1033, Blindern, N-0315 Oslo 3, Norway and b Department of Chemistry, University of Tromsø, P.B. 953, N-9001 Tromsø, Norway

Nitration of benz[a]anthracene, dibenz[a,c]anthracene, and dibenz[a,h]anthracene, using nitric acid in acetic anhydride, gave mainly isomeric mixtures of corresponding mononitro derivatives. The derivatives were purified by HPLC on silica to a purity of better than 99.5 % and identified on the basis of their 1H NMR, MS and UV data. The main isomers were 7- and 12-nitrobenz[a]anthracene, 9-nitrodibenz[a,c]anthracene, and 5- and 7-nitrodibenz[a,h]anthracene.

In order to provide standards for analytical purposes and to obtain pure reference compounds for measurements of mutagenic properties, a scheme for preparation and purification of nitrated polycyclic aromatic hydrocarbons (nitro-PAH) has been developed.1-3 This paper describes the synthesis, separation, and characterization by spectroscopic methods of the main mononitro derivatives formed by nitration of benz[a]anthracene (BaA), dibenz[a,c]anthracene (DacA), and dibenz[a,h]anthracene (DahA).

Benzanthracenes are among the more common PAH in the environment. Such compounds have been found in long-range transported aerosols 4 and 7-nitrobenz[a]an-

![NMR Spectrum Image](image-url)

Fig. 1. The 400 MHz 1H NMR spectrum of 7-nitrobenz[a]anthracene in CDCl3 at 24 °C relative to internal TMS.

0302-4369/85 $2.50
© 1985 Acta Chemica Scandinavica
thracene is reported to have been identified in a diesel particulate extract. Furthermore, nitration of BaA in acetic anhydride and in dichloromethane has been reported to give 7-nitrobenzanthracene in 25–55 % yield, with no information on other isomers. Nitration of DahA yields 9-nitro dibenz[a,h]anthracene but the structure elucidation is based on very limited data. Finally, nitration products from DacA have not been reported at all.

RESULTS AND DISCUSSION

Synthesis and purification. When benz[a]anthracene was nitrated, using nitric acid in acetic anhydride, a mixture of 6 isomeric mononitro derivatives was found. Four of the isomers were formed in very low yield; their identity has been discussed elsewhere on the basis of the

Fig. 3. The 400 MHz 1H NMR spectrum of 12-nitrobenz[a]anthracene in CDCl3 at 24 °C relative to internal TMS.
Nitroated Benzanthracenes

![Diagram of benzanthracene with nitro groups]

Fig. 4. The 400 MHz $^1$H NMR spectrum of 9-nitrodibenz[a,c]anthracene in CDCl$_3$ at 24 °C relative to internal TMS.

positional reactivity of BaA and the relations between structure and HPLC rentention time. The two main isomers, obtained in a 11:4 ratio, were partially separated on an open silica column. Rechromatography by HPLC on silica gave samples with a purity of approximately 99.9 %. Analyses by NMR and MS techniques (vide infra) proved that the predominant isomer was 7-nitrobenz[a]anthracene whereas the other one was 12-nitrobenz[a]anthracene. According to calculations of frontier electron densities these products result from electrophilic attack at the most reactive positions in BaA.

Nitration of dibenz[a,c]anthracene gave essentially three mononitro isomers. The two minor isomers, suggested to be 10-nitro- and 11-nitro-DacA, were difficult to isolate in high purity due to their instability in the presence of air and light, especially of the 10-nitro isomer. The main product was, however, stable and was obtained in a purity of better than 99.8 % when purified as described for the nitrobenz[a]anthracenes. NMR and MS analyses showed that the main product was 9-nitrodibenz[a,c]-anthracene which should be the major product from nitration of DacA according to frontier electron density calculations.

Finally, treatment of dibenz[a,h]anthracene with nitric acid gave almost exclusively two mononitro isomers in a 19:4 ratio. The isomers were partially separated by open column chromatography on silica, and highly purified by HPLC. Spectroscopic investigations revealed that the two isomers were 7-nitrodibenz[a,h]anthracene (the more abundant one) and 5-nitrodibenz[a,h]anthracene which are the most probable products from electrophilic nitration of DahA on the basis of frontier electron density calculations. A third nitro-DahA, suggested to be the 6-nitro isomer, was found in a very small amount.

$^1$H NMR spectroscopy. The main isomer from nitration of benz[a]-anthracene gave rise to the proton spectrum shown in Fig. 1. Conventional double resonance experiments revealed that the spectrum consists of one 1 proton singlet and one AB and two ABMX subspectra. The singlet substantiates that the nitro group is attached to either of the

positions 7 and 12, and to determine the substitution pattern NOE studies were carried out. The NOE difference spectrum obtained by irradiating the singlet (Fig. 2) confirms that the proton associated with this signal is situated in the proximity of one proton in each of the ABMX spin systems. Consequently, the main isomer is 7-nitrobenz[a]anthracene and the singlet is therefore due to H_{12} whereas H_{1} and H_{11} give rise to the enhanced doublets. It is also clear from the same spectrum that the enhancement is significantly larger (~50\%) for the low-field than for the high-field doublet; thus, the low-field doublet is due to H_{1} because this proton is considerably closer to H_{12} than H_{11}.^{14} A result of this conclusion is the interpretation of the spectrum included in Fig. 1, an interpretation that is in general agreement with those of the proton spectra of a variety of 7-substituted benz[a]anthracenes.^{10,15}

The minor mononitro isomer resulting from nitration of BaA gave the proton spectrum shown in Fig. 3. Decoupling experiments proved that this spectrum comprises the same type of subspectra as the spectrum of 7-NO_{2}-BaA; therefore, the minor isomer is necessarily 12-nitrobenz[a]anthracene.

The predominant nitro isomer of dibenz[a,c]anthracene gave the proton spectrum shown in Fig. 4. In order to deduce the coupling pattern homonuclear correlated spectroscopy (COSY)\textsuperscript{16–18} was carried out. In the resulting spectrum (Fig. 5) the conventional proton spectrum appears along the diagonal whereas chemical shift correlations, mediated by scalar \textsuperscript{1}H–\textsuperscript{1}H couplings, are manifested by cross-peaks which appear symmetrically with respect to the diagonal. Thus, it is evident that the proton spectrum essentially contains one singlet and

Fig. 6. Contour plot of the 400 MHz $^1$H 2D NOESY spectrum of 9-nitro dibenz[a,c]anthracene in CDCl$_3$ at 24 °C.

Fig. 7. The 400 MHz $^1$H NMR spectrum of 7-nitro dibenz[a,h]anthracene in CDCl$_3$ at 24 °C relative to internal TMS.

Fig. 8. The 400 MHz $^1$H NMR spectrum of 5-nitrodibenz[a,h]anthracene in CDCl$_3$ at 24 °C relative to internal TMS. For numbering, see Fig. 7.

three ABMX subspectra; therefore, the isomer is 9-nitrodibenz[a,c]anthracene and the singlet at 9.19 ppm is ascribed to H$_{14}$. Complete assignment of the spectrum was achieved by performing 2D nuclear Overhauser enhancement spectroscopy (NOESY).$^{19,21}$ In this experiment dipole-dipole cross relaxation between a pair of closely spaced protons results in the appearance of off-diagonal peaks which then link the corresponding diagonal resonances. The NOESY spectrum of 9-NO$_2$-DacA is depicted in Fig. 6. The most intense pairs of cross-peaks are due to contact between H$_{14}$ and the proximate protons, H$_1$ and H$_{13}$. The largest effect is expected to be between H$_{14}$ and H$_1$ which is closer to H$_{14}$ than H$_{13}$.$^{14}$

Fig. 9. Contour plot of the 400 MHz $^1$H 2D NOESY spectrum of 5-nitrodibenz[a,h]anthracene in CDCl$_3$ at 24 °C.
Fig. 10. Contour plot of the 400 MHz $^1$H COSY-90 spectrum of 5-nitrodibenz[$a,h$]anthracene in CDCl$_3$ at 24 °C.

The multiplet at 8.7 ppm is therefore ascribed to H$_1$ whereas the multiplet at 8.1 ppm is due to H$_{13}$. Subsequent combination of the remaining results of the COSY and NOESY spectra completes the assignment of the spectrum (Fig. 4). Interestingly, the COSY spectrum clearly shows long-range coupling from H$_{14}$ to a peri proton (H$_{13}$), to a proton across a bay (H$_1$), and to a proton that is connected through a conjugated chain with a zig-zag configuration. Similar couplings have also been observed in some other aromatic compounds.[22-25]

Nitration of dibenz[$a,h$]anthracene gave mainly two mononitro isomers. The predominant one gave rise to a proton spectrum (Fig. 7) that comprises one singlet in addition to two AB and two ABMX subspectra according to double resonance experiments. The singlet is due to one proton and the isomer is therefore 7-nitrodibenz[$a,h$]anthracene. The proton spectrum of the minor isomer (Fig. 8), on the other hand, consists of three 1 proton singlets and three subspectra due to one AB and two ABMX spin systems. The compound is therefore 5- or 6-nitro-DahA. In order to settle the structure 2D NOESY experiments were carried out. The NOESY spectrum (Fig. 9) clearly reveals that the proton associated with the singlet at 9.3 ppm is in the proximity of two protons of which one gives rise to the singlet at 8.7 ppm. The proton responsible for the latter singlet, on the other hand, is proximate only to one proton; consequently, H$_7$ is assigned to the singlet at 9.28 ppm and H$_6$ to the singlet at 8.73 ppm whereas the multiplet at 8.84 ppm is ascribed to H$_8$. The third singlet is thus necessarily due to H$_{14}$ which itself gives rise to two pairs of symmetric off-diagonal peaks caused by interactions with H$_1$ and H$_{13}$. The most intense cross-peaks are due to contact between H$_{14}$ and the closer proton,$^{14}$ viz. H$_1$, and the multiplet at 8.93 ppm is therefore assigned to H$_1$ whereas the doublet at 7.97 ppm is assigned to H$_{13}$. Further

Fig. 11. UV spectra in dichloromethane of 5-nitrodibenz[a,h]anthracene (A), 7-nitrodibenz[a,h]anthracene (B), 7-nitrobenz[a]-anthracene (C), 12-nitrobenz[a]anthracene (D) and 9-nitrodibenz[a,c]anthracene (E).

Table 1. Mass spectrometric fragments, in % of the base peak.

<table>
<thead>
<tr>
<th>Compound</th>
<th>M</th>
<th>M-17&lt;sup&gt;a&lt;/sup&gt;</th>
<th>M-30&lt;sup&gt;b&lt;/sup&gt;</th>
<th>M-31&lt;sup&gt;c&lt;/sup&gt;</th>
<th>M-46&lt;sup&gt;d&lt;/sup&gt;</th>
<th>M-47&lt;sup&gt;e&lt;/sup&gt;</th>
<th>M-58&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Substituent position</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-NO&lt;sub&gt;2&lt;/sub&gt;-BaA</td>
<td>100</td>
<td>65</td>
<td>71</td>
<td>4</td>
<td>47</td>
<td>87</td>
<td>93</td>
<td>peri</td>
</tr>
<tr>
<td>12-NO&lt;sub&gt;2&lt;/sub&gt;-BaA</td>
<td>72</td>
<td>7</td>
<td>100</td>
<td>23</td>
<td>38</td>
<td>90</td>
<td>56</td>
<td>bay</td>
</tr>
<tr>
<td>9-NO&lt;sub&gt;2&lt;/sub&gt;-DacA</td>
<td>34</td>
<td>7</td>
<td>100</td>
<td>31</td>
<td>24</td>
<td>58</td>
<td>41</td>
<td>bay</td>
</tr>
<tr>
<td>5-NO&lt;sub&gt;2&lt;/sub&gt;-DahA</td>
<td>100</td>
<td>65</td>
<td>100</td>
<td>28</td>
<td>20</td>
<td>60</td>
<td>84</td>
<td>peri</td>
</tr>
<tr>
<td>7-NO&lt;sub&gt;2&lt;/sub&gt;-DahA</td>
<td>81</td>
<td>7</td>
<td>100</td>
<td>26</td>
<td>44</td>
<td>96</td>
<td>99</td>
<td>bay</td>
</tr>
</tbody>
</table>

<sup>a</sup> M-<OH. <sup>b</sup> M-NO. <sup>c</sup> M-HNO. <sup>d</sup> M-NO<sub>2</sub>. <sup>e</sup> M-HNO<sub>2</sub>. <sup>f</sup> M-NOCO.

Interpretation of the NOESY spectrum combined with interpretation of the COSY spectrum of the compound (Fig. 10) results in the complete assignment included in Fig. 8. The COSY spectrum also reveals the presence of various long-range couplings; the most predominant ones are between peri protons (H<sub>6</sub>-H<sub>7</sub> and H<sub>13</sub>-H<sub>14</sub>), between bay protons (H<sub>1</sub>-H<sub>14</sub>), and through a zig-zag bond system (H<sub>6</sub>-H<sub>14</sub> and H<sub>6</sub>-H<sub>12</sub>).

**Mass spectrometry.** The mononitro derivatives gave mass spectra that contained fragments due to fragmentation processes typical for this class of compounds<sup>3</sup> (Table 1). In accordance with previous observations<sup>3</sup> M-<OH and M-HNO fragments are found simultaneously only in the spectra of isomers with the nitro group in a bay region. The mass spectra therefore confirm the structure elucidation carried out on the basis of the NMR experiments,

**UV spectroscopy.** The UV spectra of the nitrobenzanthracenes and nitrodibenzenanthracenes are in accordance with the assigned structures. Thus, the long wavelength absorption in the 370–390 nm region in the spectrum of 5-nitrodibenzen[a,h]anthracene (Fig. 11) is not present in the spectrum of 7-nitrodibenzen[a,h]anthracene where the nitro group is in a bay region. This seems generally to be the case with isomeric nitro-substituted polycyclic aromatic hydrocarbons.<sup>1</sup> The same trend, although less marked, was seen in the spectra of 7- and 12-nitrobenzen[a]anthracene (Fig. 11).

**EXPERIMENTAL**

The instruments used have been described elsewhere.<sup>1</sup> *Synthesis and purification.* The nitration temperature was 50 °C. The conditions were selected in order to obtain maximum yield of mononitro derivatives. Nitration and crude purification was carried out essentially as previously described.<sup>1,3</sup> On open silica columns the nitrobenz[a]anthracenes and the nitrodibenzen[a,h]anthracenes were eluted with 25 % chloroform in hexane, while the nitrobenz[a,c]anthracenes were eluted with 25 % dichloromethane in hexane. The chloroform was stabilized with 0.5 % ethanol and the dichloromethane contained 0.1 % methanol. The mononitro fractions were obtained in 70–85 % yields. Additional experimental details are summarized in Table 2. Preparative HPLC purifications of each isomer was performed on a 250×7.8 mm Hypersil 3 μm silica column, with 2.5 % dichloromethane in hexane.

<sup>1</sup> *H NMR spectroscopy.* The spectra were run in 5 mm NMR tubes on samples which were 0.2–0.5 % by weight in deuteriochloroform (99.9 %). The ordinary spectra were run as previously described.<sup>1</sup> The NOESY spectra were obtained using a (90°-t<sub>m</sub>-90°-t<sub>m</sub>-90°-t<sub>m</sub>)<sub>n</sub> sequence with a mixing time t<sub>m</sub> of 1–2 s and with pre-irradiation of the different proton chemical shift frequencies for 5 s. After collecting 8 scans, preceded by 2–4 dummy scans, at one selected irradiation frequency the FID was stored and the irradiation frequency was 2.03 ppm.

Table 2. Synthesis and crude purification of mononitro benzanthracenes and dibenzanthracenes on Merck Silica 60 (0.063–0.200 mm).

<table>
<thead>
<tr>
<th>PAH</th>
<th>Molar ratio HNO₃/PAH</th>
<th>Reaction time (h)</th>
<th>Column size (cm)</th>
<th>Applied amount (mg)</th>
<th>Fraction (ml)</th>
<th>Nitro isomers</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaA</td>
<td>7.5</td>
<td>114</td>
<td>28x4.5</td>
<td>165</td>
<td>1400–1750</td>
<td>12-nitro</td>
<td>122–128 (12-nitro)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1750–3000</td>
<td>12-nitro and 7-nitro</td>
<td>160–163 (7-nitro)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3000–4000</td>
<td>7-nitro and minors</td>
<td>158–161 (9-nitro)</td>
</tr>
<tr>
<td>DacA</td>
<td>10</td>
<td>65</td>
<td>28x1.5</td>
<td>18</td>
<td>1'2–170</td>
<td>9-nitro and minor</td>
<td>224–226 (7-nitro)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>210–310</td>
<td>minor</td>
<td>236–240 (5-nitro)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>800–1400</td>
<td>7-nitro</td>
<td></td>
</tr>
<tr>
<td>DahA</td>
<td>12</td>
<td>52</td>
<td>28x3.5</td>
<td>75</td>
<td>1700–2600</td>
<td>5-nitro and minor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.58 (1H, m, J 0.6 Hz, J 8.2 Hz), 8.68 (1H, m, J 9.19 (1H, s))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgements. Financial support from The Norwegian Council for Scientific and Industrial Research (NTNF), The Norwegian Research Council for Science and the Humanities (NAVf) and from Norges Vassdrags og Elektrisitetsvesen-Statenskraftverkene is gratefully acknowledged. Thanks are also due to Unni Bingen, Liv Larsen and Tore Skjetne for skillful technical assistance.

REFERENCES

25. Sydnes, L.K. and Skjetne, T. *To be published.*

Received February 8, 1985.