
Product Ion Studies of Diastereomeric Benzo[ghi]fluoranthene Tetraols by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Post-Source Decay

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The product ion formation characteristics of the four diastereomeric tetrahydroxy benzo[ghi]fluoranthene compounds formed by hydrolysis of the syn and anti diastereomers of trans-3,4-dihydroxy-5,5a-epoxy-3,4,5,5a-tetrahydrobenzo[ghi]fluoranthene are studied using matrix-assisted laser desorption/ionization and post-source decay (PSD) to determine a correlation between the fragmentation characteristics of these tetraols and the structures of the diol-epoxide diastereomers from which they are hydrolyzed. The tetraols formed by the trans ring opening of the diol epoxides during hydrolysis yield product ion spectra specific for the syn and anti configurations of their precursor diol epoxides. All four diastereomeric tetraols form product ions by the losses of one and/or two water molecules in varying proportions when lithium-cationized molecule ions (m/z 301) are selected for PSD product ion analysis. The differences in the PSD spectra of these four Li^+ -cationized molecules are rationalized in terms of a water loss mechanism that involves the 1,2 elimination of a hydrogen atom and hydroxyl group that are cis with respect to each other on adjacent carbons. (J Am Soc Mass Spectrom 2001, 12, 376–380) © 2001 American Society for Mass Spectrometry

Polynuclear aromatic hydrocarbons (PAH) are environmental carcinogens to which human exposure is widespread [1]. These compounds are formed as a result of the incomplete combustion of a number of different organic materials, including tobacco and fossil fuels. Metabolic activation of these compounds may lead to the formation of diol epoxides that may bind covalently to DNA in route to tumor formation. The analysis of these DNA adduct species in humans is difficult because DNA is approximately 0.1%

of the mass of human tissue and very few nucleic acids need to be modified by carcinogens to initiate tumor formation (perhaps less than 1 adduct in 10^7 bases) [2].

Analytical methods currently used to measure PAH-DNA adducts formed in humans, e.g., ^{32}P -postlabeling [3], immunoassays [4], and fluorescence [5], are very sensitive but provide limited structural information. Therefore such methods may be used to estimate total adduct levels following an incidence of exposure to a complex mixture of carcinogens, but information regarding exposure to specific PAH may not be acquired. As a result, alternate strategies for determining exposure to specific PAH based on the use of hemoglobin as surrogate for DNA are being investigated [6]. PAH tetraols hydrolyzed from hemoglobin in-vitro or in laboratory animals have been analyzed using a variety

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of analytical techniques, many of which are based on fluorescence and immunochemical detection [7–9].

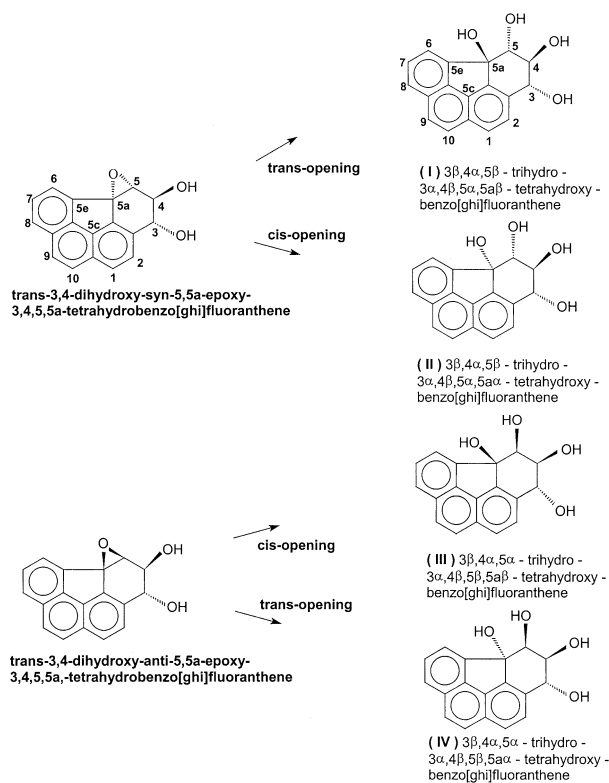
Strategies for the analysis of specific PAH based on gas chromatography mass spectrometry, using negative ion chemical ionization (NICI) and single-ion monitoring, are attractive for the analysis of PAH tetraols because of the sensitivity and specificity of the overall method [10–12]. However, the NICI mass spectra of the derivatized tetraols do not permit differentiation of diastereomers. The identification of different diastereomers requires co-chromatography with standard compounds. The ability to identify different tetraol diastereomers (that may be produced in studies of PAH metabolism) using tandem mass spectrometry is desirable because synthetic tetraol standards would not be needed in these experiments. Therefore the development of analytical methods capable of detecting and differentiating tetraol diastereomers based on tandem mass spectrometry at low levels merits investigating.

Benzo[ghi]fluoranthene is of interest because this PAH has many known sources and significant levels of this PAH have been detected in urban atmospheres [13]. The ability to differentiate diastereomeric tetraols of benzo[ghi]fluoranthene is desirable because the configuration of the hydroxy groups attached to the tetraol is related to the stereochemistry of its PAH diol-epoxide precursor. The ability to detect low levels (femtomole quantities) of PAH tetraols with stereochemical information may permit the association of different PAH-diol epoxide diastereomers with their carcinogenic potential.

Experimental

The preparation and ¹H NMR structural characterization of these tetraols used in this study and their diol-epoxide precursors is described elsewhere [14, 15]. Tetraols **I** and **II** were prepared by hydrolysis of trans-3,4-dihydroxy-syn-5,5a-epoxy-3,4,5,5a-tetrahydrobenzo[ghi]fluoranthene. Tetraols **III** and **IV** were generated by hydrolysis of trans-3,4-dihydroxy-anti-5,5a-epoxy-3,4,5,5a-tetrahydrobenzo[ghi]fluoranthene. The hydrolysis reaction of the syn- and anti-diol epoxides is illustrated in Scheme 1. 2,6-Dihydroxyacetophenone (2,6-DHA) and α -cyano-4-hydroxycinnamic acid (CHCA) were used as matrices in this investigation. These matrices were purchased from Aldrich (Milwaukee, WI) and used without further purification.

Matrix-assisted laser desorption/ionization and post-source decay (MALDI-PSD) spectra were acquired with a PerSeptive Biosystems, Voyager DE-RP mass spectrometer (Framingham, MA) at the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry in St. Louis, MO. The instrument was operated in the continuous ion extraction mode with a total acceleration potential of 25 kV. A nitrogen laser (337 nm, 20 kW peak laser power, 5 ns pulse width) was used to induce desorption and ionization. All spectra were acquired in the positive ion mode. The



Scheme 1. The structures and numbering of the syn- and anti-benzo[ghi]fluoranthene diol epoxides and the tetraols produced by their hydrolysis.

tetraols used in this study were dissolved in methanol to give final concentrations of 2–4 pmol/ μ L⁻¹. The matrix was prepared as a 1 mg/mL solution in 2:1 CH₃CN:H₂O that was 0.1% in trifluoroacetic acid. Aliquots of tetraol and matrix solutions were combined in different ratios and 1 μ L of the resulting mixture was applied to the sample probe for analysis. The matrix solutions were made 0.025 M in LiNO₃ for experiments where the generation of Li⁺-cationized molecule ions was desirable. The absolute quantity of analyte applied to the laser probe in the individual sample preparations was varied from 0.5 to 2 pmol. The samples were allowed to dry under ambient conditions before introduction into the mass spectrometer. The product ion formation characteristics of these Li⁺-cationized tetraols were compared using 0.5 to 1 pmol per sample preparation at laser power densities near the threshold for observation of product ions. Spectra were acquired by selecting the *m/z* 301 ion for product ion analysis and then increasing the laser power until the product ions formed by water loss were observed. The matrix:analyte ratio used for these analyses was varied from 300 to 5 \times 10⁴:1. The resolution with which precursor ions could be selected for product ion analysis was approximately 25–50. PSD spectra were acquired with a single reflector voltage setting. The background signal level was determined by analyzing the matrix alone in the same mass window(s) in which the cationized molecule

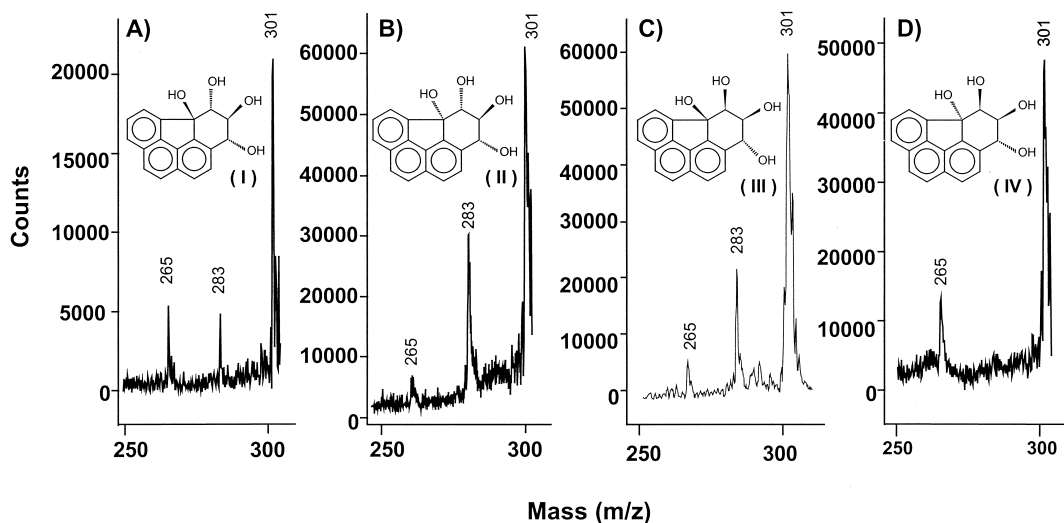


Figure 1. MALDI PSD product ion spectra of the Li^+ -cationized molecule ion at m/z 301 derived from (A) $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5\beta$ -tetrahydroxy benzo[ghi]fluoranthene (I), (B) $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (II), (C) $\pm 3\beta,4\alpha,5\alpha$ -trihydro- $3\alpha,4\beta,5\beta,5\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (III), and (D) $\pm 3\beta,4\alpha,5\alpha$ -trihydro- $3\alpha,4\beta,5\beta,5\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (IV).

ions were selected for PSD analysis. Normally, 20–30 laser shots were averaged to produce a PSD spectrum. Three or four 20–30 laser shot spectra were acquired from each 1 μL sample preparation. A minimum of two sample preparations were analyzed for the purpose of comparing abundances of product ions formed by water loss. Ion abundance ratios were calculated using peak heights. Raw data were acquired with a Tektronix TDS 520A oscilloscope and subsequently transferred to PC equipped with GRAMS/386 software for data processing.

Results and Discussion

Molecule Ion Formation in MALDI

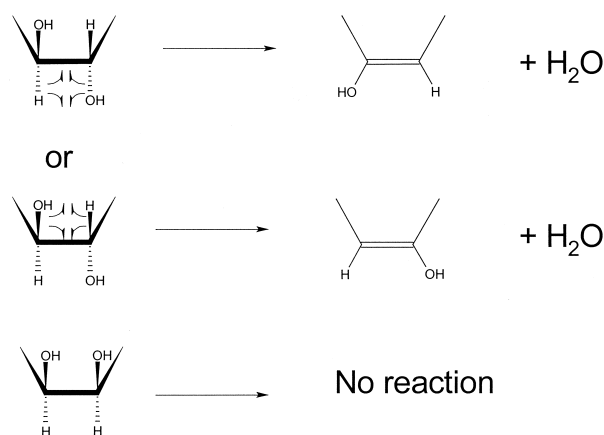
The structures of the four benzo[ghi]fluoranthene tetraol diastereomers used in this study differ due to the configuration of hydroxyl groups and hydrogens attached to carbons 3, 4, 5, and 5a (Scheme 1). Our initial experiments were carried out to establish experimental conditions that promoted the strongest molecule ion signals. Several matrices were tested as vehicles for the desorption and ionization of these diastereomers, but in no instance was a protonated molecule ion at m/z 295 observed. Only fragment ions formed by the losses of H_2O and CO were detected. This fragmentation in the hydroxylated aromatic ring of the PAH observed in the molecular weight spectra of these tetraols is consistent with that observed in the product ion spectra of the BH_2^+ ions of diol-epoxide nucleic acid adducts generated by MALDI and fast-atom bombardment (FAB) ionization [16, 17]. The inability to generate protonated molecule ions of these tetraols appears consistent with earlier MALDI studies of dibenzo[a,i]pyrene PAH diols, where at least five matrices were tested for the deter-

mination of these tetraols, but only α -cyano-4-phenylcinnamic acid (PCC) promoted the formation of a protonated molecule ion [18].

In the next series of experiments we tested several lithium and sodium salts as cationizing agents for molecule ion generation in the MALDI analyses of these tetraols. Of those salts tested, LiNO_3 appeared to give the most consistent cationized molecule ion signal, $(M + \text{Li})^+$, at m/z 301. Two matrices, CHCA and 2,6-DHA, were found to support formation of cationized tetraol molecule ions. The Li^+ -cationized tetraols gave similar PSD spectra using either matrix at laser power densities near threshold for fragment ion formation.

MALDI-PSD Studies of $(M + \text{Li})^+$ Tetraols

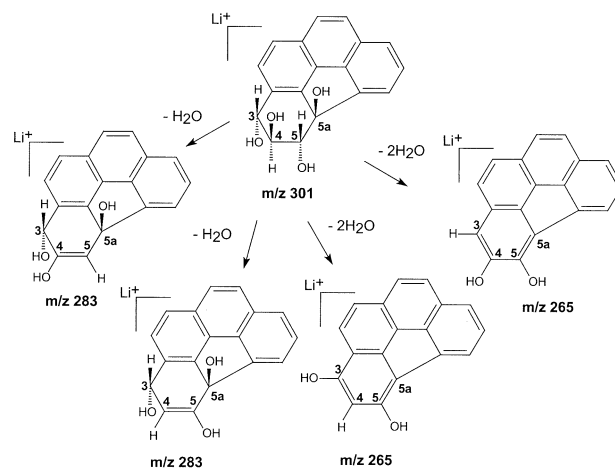
Product ion studies of the lithiated tetraol diastereomers at m/z 301 were conducted to determine if their PSD spectra could be correlated with the structure of their diol-epoxide precursor. Their product ion spectra are presented in Figure 1. All four lithiated diastereomers produce product ions formed by the loss of one or two H_2O groups. No product ions formed by any other neutral loss are observed at laser power densities near threshold for fragment ion formation. The lithiated molecule ion from $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5\beta$ -tetrahydroxy benzo[ghi]fluoranthene (I), fragments to yield product ions at m/z 265 and 283 in approximately equal abundances (Figure 1A). The product ion ratio (m/z 265/283) is 1.0 ± 0.1 for the average of eight 20–30 laser shot spectra. The $(M + \text{Li})^+$ ions from $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (II) and $\pm 3\beta,4\alpha,5\alpha$ -trihydro- $3\alpha,4\beta,5\beta,5\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (III) lose one water molecule predominantly to form a major product ion at



Scheme 2. Proposed mechanism of water loss from the Li⁺-cationized tetraols used in this study.

m/z 283 (Figures 1B and 1C, respectively). The (M + Li)⁺ ion from $\pm 3\beta,4\alpha,5\alpha$ -trihydro- $3\alpha,4\beta,5\beta,5a\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (IV) produces a single product ion at *m/z* 265 upon PSD (Figure 1D). These water losses may bring about the formation of one or two double bonds between carbons 3, 4, 5, and 5a (Scheme 1). The ability of a lithiated tetraol to lose a water molecule is dictated by the cis/trans orientation of hydrogen atoms and hydroxyl groups on adjacent carbon atoms (Scheme 2). The simplest water loss mechanism that is consistent with all the product ion spectra in Figure 1 involves a 1,2 elimination of a hydrogen and a hydroxyl group that are cis with respect to each other. Evidence for such a 1,2 elimination of water has been observed previously in product ion spectra of Li⁺-cationized unsaturated fatty alcohols [19]. Double bond formation does not appear to be possible between adjacent carbons that are bonded to hydroxyl groups that are cis with respect to each other. The generality of this mechanism is illustrated in the discussion of the individual product ion spectra below.

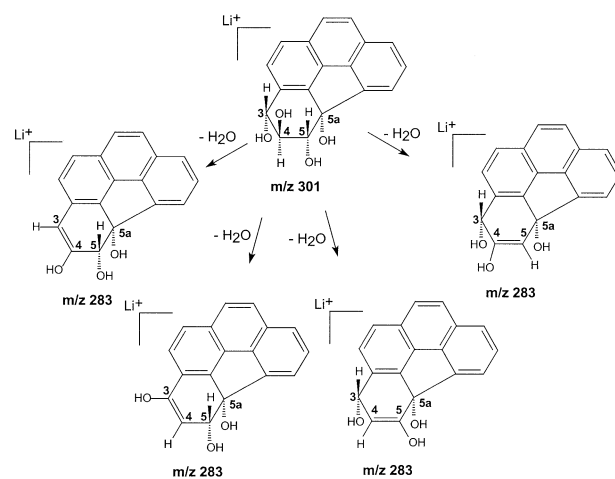
The formation of the *m/z* 265 ion in the PSD analysis of tetraol I is likely brought about the 1,2-elimination of water to form double bonds between carbons C3 and C4 and carbons C5 and C5a to form a fully conjugated dihydroxy benzo[ghi]fluoranthene (Scheme 3). The 1,2 elimination of water between carbons C4 and C5 produces the product ion at *m/z* 283. Once a double bond is formed between carbons C4 and C5, the loss of another water is not favored because the energy required to form two double bonds between three adjacent carbons is not available at laser power densities employed for these studies. Once a double bond is formed between carbons C3 and C4 or C5 and C5a, a second water loss occurs to form a fully conjugated molecule. Competition between the formation of *m/z* 265 and 283 in the fragmentation of tetraol I is probably determined by the first 1,2 elimination of water. If the first elimination occurs between either carbons C3 and C4 or C5 and C5a, then a second water loss will occur and *m/z* 265 is formed. If water loss



Scheme 3. Proposed mechanism of fragmentation of the Li⁺-cationized $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5a\beta$ -tetrahydroxybenzo[ghi]fluoranthene ion at *m/z* 301.

occurs between C4 and C5 first to form a double bond, then a second water loss will not occur because the formation of a fully conjugated dihydroxy benzo[ghi]fluoranthene is not possible. Thus an ion at *m/z* 283 is formed. These mechanistic considerations are consistent with (and supported by) the product ions observed in the PSD spectra of tetraols II–IV discussed below.

The (M + Li)⁺ ions formed from tetraols II and III undergo loss of one water to form a product ion at *m/z* 283 predominantly because the hydroxyl groups on carbons 5 and 5a are cis with respect to each other for both molecules (tetraol II, Scheme 4). Consequently, a product ion formed by loss of two waters is never very abundant at the laser energies employed for comparison of PSD spectra associated with these Li⁺-cationized diastereomers. The product ion ratios (*m/z* 265/283) were found to be 0.1 ± 0.1 for the average of eight 20–30 laser shot spectra for both tetraols II and III.



Scheme 4. Proposed mechanism of fragmentation of the Li⁺-cationized $\pm 3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5a\alpha$ -tetrahydroxybenzo[ghi]fluoranthene ion at *m/z* 301.

Tetraols **II** and **III** cannot be distinguished by PSD analysis of their corresponding lithiated molecule ions. The observation of m/z 265 ion from tetraols **II** and **III** would require the formation of two double bonds, one between carbons C3 and C4, and the other between carbons C4 and C5. Double bond formation is possible between either carbons C3 and C4 or carbons C4 and C5 for tetraol **II** (Scheme 3). In the case of tetraol **III**, double bond formation would be favored only between carbons C3 and C4.

The $(M + Li)^+$ ion of tetraol **IV** yields a single product ion at m/z 265 because this diastereomer is not capable of forming a double bond between carbons C4 and C5. The two hydroxyl groups (and two hydrogen atoms) bonded to carbons C4 and C5 are cis with respect to each other. Double bond formation is possible between carbons C3 and C4 and carbons C5 and C5a. The ion formed by loss of one water molecule at m/z 283 is only barely perceptible, if present at all, in the PSD spectra of the tetraol **IV** acquired at any laser power density used in this study.

Conclusion

The product ion formation characteristics of the $(M + Li)^+$ molecule ions of the four diastereomeric benzo[ghi]fluoranthene tetraols were investigated for the purpose of correlating the tetraol PSD spectra with the structure of the diol-epoxide diastereomer from which the tetraols are hydrolyzed. The tetraol formed by the trans ring-opening of each diol-epoxide diastereomer gives a product ion spectrum that is different from those of the other three tetraols, $3\beta,4\alpha,5\beta$ -trihydro- $3\alpha,4\beta,5\alpha,5a\alpha$ -tetrahydroxy benzo[ghi]fluoranthene (**I**), derived from the hydrolysis of trans-3,4-dihydroxy-syn-5,5a-epoxy-3,4,5,5a-tetrahydrobenzo[ghi]fluoranthene, produces equal abundances of m/z 283 and 265 when the lithiated molecule ion is subjected to PSD analysis. $3\beta,4\alpha,5\alpha$ -Trihydro- $3\alpha,4\beta,5\beta,5a\alpha$ -tetrahydroxybenzo[ghi]fluoranthene (**IV**), derived from the hydrolysis of trans-3,4-dihydroxy-anti-5,5a-epoxy-3,4,5,5a-tetrahydrobenzo[ghi]fluoranthene, produces a single product ion at m/z 265 when the lithiated molecule ion is subjected to PSD analysis. The mechanism of fragmentation involves a 1,2 elimination of a hydrogen atom and hydroxyl group that are cis with respect to each other. These results suggest other tetraols formed by the trans ring-opening of other PAH diol epoxides (during hydrolysis) may serve as biological markers for the attachment of particular diol-epoxide diastereomer to the DNA of a laboratory animal dosed with a PAH of known structure. Such studies should be feasible using rats if substitution levels of one adduct in 10^7 nucleotides are achieved. At this level of substitution, 20 mg of

DNA isolated from a single rat liver would provide 6 pmol of all four tetraol diastereomers upon hydrolysis. Questions concerning PAH metabolism and the DNA binding of specific diastereomers could be answered with these quantities of sample using MALDI after liquid chromatography separation.

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