

SOLVOLYSIS OF THE BAY-REGION DIOL-EPOXIDE OF BENZ[a]ANTHRACENE

A mass spectrometric technique to study the adduct

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1. Introduction

Polynuclear aromatic hydrocarbons (PAH) are a group of potent environmental carcinogens [1,2], that require cell-mediated activation before they can react with cellular macromolecules [3–6]. Bay-region diol-epoxides have been implicated as the possible intermediates in the carcinogenic or mutagenic process [7–11]. The accepted hypothesis for this metabolic activation entails metabolic transformation of an angularly fused benzo-ring of a PAH to a *trans*-dihydrodiol intermediary metabolite followed by enzymatic epoxidation of the bay-region double bond to form the ultimate bay-region diol-epoxide metabolite. An example of this diol-epoxide is 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene (I, fig.1). The mutagenicity of this compound is at least 10-times more potent than its corresponding K-region oxide [12] (i.e., the 5,6-oxide). The 3,4-dihydrodiol of benz[a]anthracene, presumably the precursor metabolite to the bay-region diol-epoxide, was found to be most tumorigenic among all the benz[a]anthracene dihydrodiols tested [13]. This is consistent with and supportive of the bay-region hypothesis of PAH carcinogenesis.

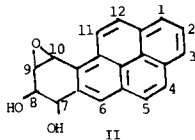
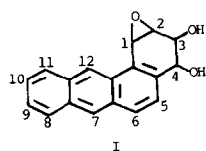


Fig.1. Bay-region diol-epoxides of benz[a]anthracene (I) and benz[a]pyrene (II).

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The bay-region diol-epoxide is uniquely different from other epoxides of PAH in that it is an aliphatic epoxide as opposed to an arene oxide and that it has notably a higher tendency to form a bay-region carbonium ion. The chemical reactivity of epoxides at various positions of a PAH can be predicted in terms of the theoretical reactivity indices based on a quantum mechanical treatment of the carbonium ion formation process [8,14]. Perturbational molecular orbital calculations also allow predictions of product structures formed during solvolysis and nucleophilic addition to the PAH epoxides [15,16]. For instance, such calculations indicate oxirane ring opening at C₁ and C₁₀ for I and 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenz[a]pyrene (II, fig.1), respectively. Experimental verifications of this theoretical prediction for II has been achieved by NMR studies of the solvolytic products [17] and by observing the proper acetonide formation for the products of the nucleophilic reaction of thiolate anion with II [18]. We now report a very efficient mass spectrometric method for elucidating the nucleophilic addition to I. This method can be generally applicable in studying the structures of the adducts for other bay-region diol epoxides. The methodology is particularly suited for probing the adducts in metabolism mixtures.

2. Materials and methods

A sample of 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene (I) was obtained from the Chemical Repository of the National Cancer Institute. This sample has the *anti* configuration in which the epoxy group is *trans* to the C₄ hydroxyl

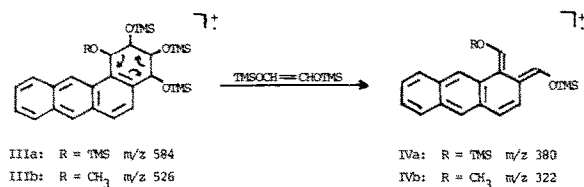


Fig.2. Retro-Diels-Alder fragmentation of bay-region derivatives.

group. The 1,2,3,4-tetrahydrotetraol of benz[a]-anthracene was prepared by hydrolysis of I in aqueous pyridine. This tetrahydrotetraol was subsequently converted to the trimethylsilyl ether derivative (IIIa, fig.2) by treatment with *N,O*-bis-(trimethylsilyl)-trifluoroacetamide. Gas chromatography-mass spectrometric analysis (GC-MS) of this compound gave the mass spectrum shown in fig.3a.

Methanolysis of the diol epoxide I was effected by heating 100 μ g of I in 200 μ l spectral grade methanol at 60°C for 4 h. After solvolysis, the solvent was removed by evaporation in a stream of nitrogen. The product was then trimethylsilylated (IIIb, fig.2) by *N,O*-bis-(trimethylsilyl)-trifluoroacetamide and analysed by GC-MS. The mass spectrum is shown in fig.3b. Similarly, a sample of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene (the *anti* form from the Chemical Repository of NCI) was methanolized and studied by GC-MS.

Mass spectrometric analysis was done on a Hewlett-Packard model 5985 GC-MS instrument. The gas chromatograph was a Hewlett-Packard model 5840 unit equipped with a 6 ft \times 1/8 in column of 3% OV-17 on Gas-Chrom Q, 100–120 mesh. The mass spectrometer was operated in the electron impact ionization mode at an ionization voltage of 70 eV.

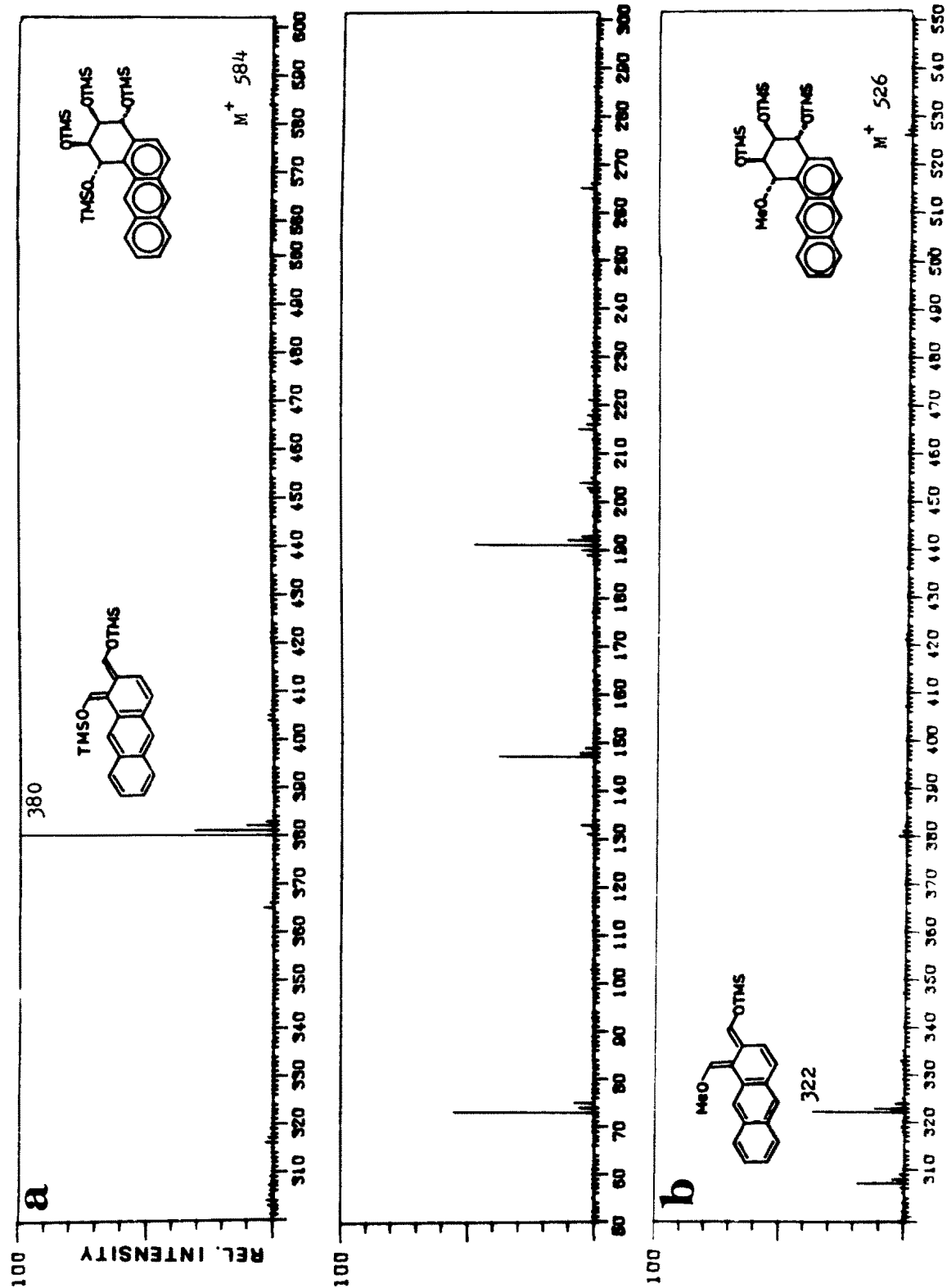
3. Results and discussion

The mass spectrum (fig.3a) of the trimethylsilyl ether derivative of the 1,2,3,4-tetrahydrotetraol (IIIa) shows a molecular ion at m/z 584. The base peak is at m/z 380, indicative of a facile retro-Diels-Alder [19] fragmentation ion (IVa, fig.2). The prominent ion at m/z 191 (~50%) is a di-(trimethylsilyloxy) ion reminiscent of a vicinal dihydrodiol moiety present at a non K-region location of the polycyclic skeleton. We had noted this feature in [20]. The crucial pattern

in this mass spectrum is the retro-Diels-Alder fragment. Since this fragment retains the substituents at C₁, it is useful for determining the orientation of the oxirane ring opening process for the bay-region epoxide. If a nucleophile should attack the bay-region epoxide at C₁ as predicted by the bay-region hypothesis of carcinogenesis, the retro-Diels-Alder fragment should reflect the formation of such an adduct at that particular site of the molecule.

The product from the methanolysis of the benz[a]-anthracene bay-region diol-epoxide (I) was consonant with the bay-region hypothesis. As shown in its mass spectrum (fig.3b), this compound has the expected molecular ion at m/z 526 signifying the introduction of a methoxyl group. The retro-Diels-Alder fragment was found at m/z 322 which clearly indicated that the attack of methanol occurred at the C₁ position. This is in accord with the molecular orbital calculations mentioned earlier. The base peak at m/z 133 is attributed to a methoxy-trimethylsilyloxy ion similar to the m/z 191 ion for the di-(trimethylsilyloxy) fragment discussed in [21]. Thus the mass spectral features described here provide a very efficient means to elucidate the attack of bay-region diol-epoxides by nucleophiles.

We have also investigated the methanolysis of the 1,2-dihydrodiol-3,4-epoxide of benz[a]anthracene, a non bay-region diol-epoxide analogue. The mass spectrum (fig.3c) of the methanolized product revealed that the methoxyl group was introduced to C₄ of the epoxide. This is again consistent with the theoretical prediction [9] that the benzylic position at C₄ would be more reactive than C₃. Theoretical calculations also indicated that the bay-region diol-epoxide (I) should be more reactive than the non bay-region 1,2-dihydrodiol-3,4-epoxide. This is in parallel with the observed mutagenicity of the corresponding saturated ring A epoxides [13]. In our experiments, we observed that after 2 h, the bay-region diol-epoxide underwent ~40% conversion to its methanolate while the non bay-region analogue underwent <0.1% conversion. After 5 h, there was >70% conversion for the former but only ~30% conversion for the latter. These experimental data clearly show that the C₁ position of the benz[a]anthracene bay-region diol-epoxide is particularly reactive. The mass spectrometric technique presented here is particularly suited for the characterization of diol-epoxide biomolecular adducts postulated in the carcinogenic process of these compounds.



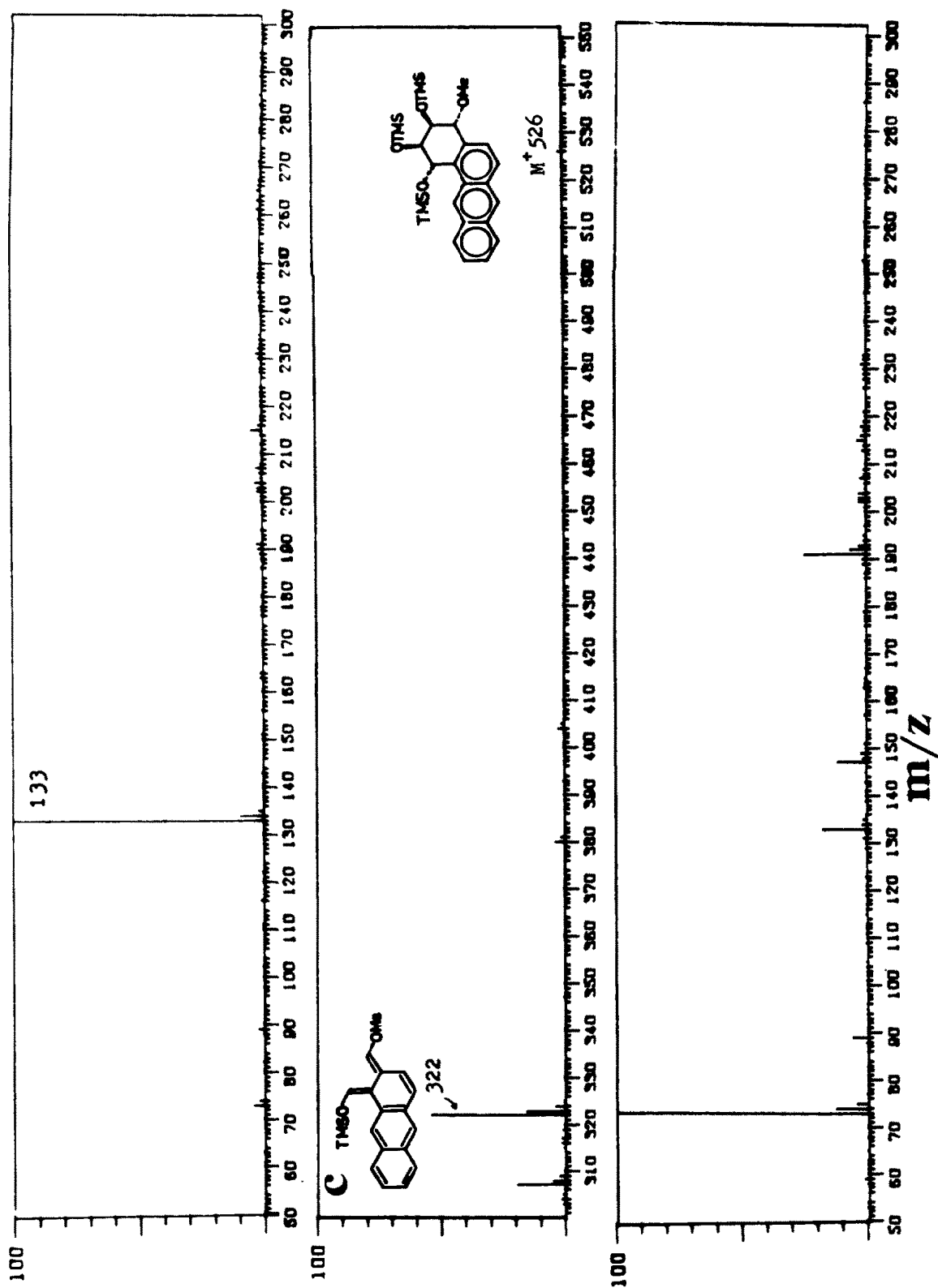


Fig.3. (a) Mass spectrum of the TMS derivative of benz[a]anthracene-1,2,3,4-tetrahydrotetraol. (b) Mass spectrum of the methanolysis product of the bay-region diol-epoxide of benz[a]anthracene. (c) Mass spectrum of the methanolysis product of the non bay-region diol-epoxide analogue.

Acknowledgements

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