Identification of Benzo(a)pyrene Metabolites by Gas Chromatograph-Mass
Spectrometer

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ABSTRACT

A gas chromatograph-mass spectrometer was used in an attempt to achieve rapid separation and accurate identification of benzo(a)pyrene and its synthesized derivatives. All derivatives, after being trimethylsilylated, were developed on Dexsil-300 and OV-1 columns. The seven diols and four stereoisomeric 7,8,9,10-tetraols studied were separated successfully. The separation of the 12 phenols was unsatisfactory; 8- and 11-isomers appeared separately but the other 10 isomers made 3 peaks on the OV-1 column. Among the five derivatives reported to be present in animal tissues, 6-, 7-, and 9-phenols were separated, but 1- and 3-phenols were fused on the Dexsil-300 column. Quinones were converted to related dihydroxyl derivatives under silylation. The chromatographic separation of four of the six dihydroxyl derivatives was successful on the OV-1 column, but the 6,12- and 7,10-isomers remained in a single peak. The two diol-epoxides were unstable under silylation and therefore were detected by their breakdown products, 7,8,9-trihydroxy-7,8-dihydrobenzo(a)pyrene and tetaols.

Data are listed on the mass spectra and retention times of all benzo(a)pyrene derivatives studied.

INTRODUCTION

Polycyclic aromatic hydrocarbons are considered to become carcinogenic after oxidation by microsomal mixed-function oxygenases (4, 6, 17). An environmental carcinogen, BP, is metabolized to epoxides, diols, phenols, and dihydroxyl compounds (17). The carcinogenic activities of some of these BP derivatives have been tested on mouse skin (11, 12, 22) and by i.p. injection in newborn mice (7, 8). Among the BP derivatives reported, diol-epoxide 2 (8), trans-7,8-diol (7), 7,8-oxide (12), and 2-OH-BP (22) are more carcinogenic than is the parent hydrocarbon, BP, whereas 4,5-oxide (12), diol-epoxide 1 (8), and 11-OH-BP (22) are moderately or weakly carcinogenic. This information on proximate or ultimate carcinogens is valuable for an understanding of the mechanism of chemical carcinogenesis.

During the past 2 decades, several BP metabolites, e.g., 1,2-, 4,5-, 7,8-, and 11,12-diols; 4,5-oxide; 1,6-, 3,6-, and 6,12-dihydroxy compounds; 1,6- and 3,6-quinones; and phenols, have been detected or tentatively identified in biological materials by conventional methods (2, 3, 16). Recently, high-pressure liquid chromatography has been introduced into the study of chemical carcinogenesis, and 4,5-, 7,8-, and 9,10-diols, 1,6-, 3,6-, and 6,12-quinones, 1-, 3-, 7-, and 9-OH-BP's, and 4,5-oxide have been detected as metabolites of BP (5, 13-15). The triol and tetaols of BP have been separated as metabolites of trans-7,8-diol (20, 26). Certainly, high-pressure liquid chromatography is a useful tool for the separation and quantification of BP metabolites; however, it cannot provide information on molecular structure.

Recently, one of the authors was successful in separating 3-MC-related compounds by a combined GC-MS (19). In the present study, we applied BP and 38 synthesized derivatives (12 phenols, 6 quinones, 6 dihydroxyl compounds, 7 diols, 4 stereoisomeric tetaols, 2 diol-epoxides, and an epoxide) to this system, in an attempt to establish a method of accurate identification and separation of BP metabolites from tissues.

MATERIALS AND METHODS

Chemicals. BP was purchased from Sigma Chemical Co., St. Louis, Mo. Its derivatives, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, and 12-OH-BP's; 1,6-, 3,6-, 6,12-, 7,10-, 4,5-, and 11,12-quinones; cis-4,5-, trans-4,5-, cis-7,8-, trans-7,8-, and trans-9,10-diols; diol-epoxides 1 and 2; and 4,5-oxide, were kindly supplied by the National Cancer Institute Carcinogenesis Research Program, NIH, Bethesda, Md. The stereoisomeric 7,8,9,10-tetraols were prepared in our laboratory from diol-epoxides 1 and 2 by hydrolysis in 10% tetrahydrofuran and 0.09 M KCl at 37° for 1 hr, according to the method of Yagi et al. (25). In the procedure, diol-epoxide 1 was hydrolyzed to tetaols cis-1a (83%) and trans-1a (17%), and diol-epoxide 2 was converted to tetaols cis-2a (38%) and trans-2a (62%).

Isomeric quinone and lithium tetrahydroaluminate were each heated in ether under reflux for 30 min. This reduction yielded 1,6-, 3,6-, 6,12-, 7,10-, 4,5-, and 11,12-dihydroxy-BP's. The crude products obtained by reduction of 11,12-quinone were developed on thin-layer silica gel chromatography in benzene: ethanol (95:5), and 2 spots of 11,12-diols were obtained with Rf's 0.17 and 0.20. A minor diol in the first-running spot was identified as cis-configuration and the major slow-running product as trans-configuration because, in the first, on thin-layer chromatography cis-4,5- and cis-7,8-diols were detected faster than were trans-4,5- and trans-7,8-diols, respectively; in the second, the major diol of a pair of diols obtained by reduction of 4,5-quinone was identified as trans-configuration.

The BP derivatives were silylated at 65° for 15 min with pyridine:N,O-bis(trimethylsilyl)acetamide:trimethylchlorosilane (2:2:1) in N2 gas-sealed glass tubes.

Analytical System. A combined system of a JEOL JGC-20K...
gas chromatograph, a JEOL JMS D-300 mass spectrometer, and a mass data analysis system JEOL JMA-2000 were used at an ionization current of 300 μA, an ionization voltage of 25 eV, and a mass range of 50 to 650. Each analytical standard (5 nmol/μl) was mixed with internal standards of benz(a)anthracene and dibenz(a,h)anthracene. They were applied to gas chromatograph columns of OV-1 (2%) and Dexsil-300 (6%), which were packed in a glass tube (1 m or 2 m x 2 mm) together with Chromosorb W. The gas chromatograph was operated at a constant temperature of 250° for OV-1 and 290° for Dexsil-300. The carrier gas was helium, and the flow rate was about 20 ml/min. Operation of the mass spectrometer was started 0.5 to 2 min after injection of the sample and continued for 10 to 20 min at 4-sec scanning intervals.

RESULTS

Stability of BP Derivatives during Analysis. All the phenols were stable under these analytical conditions, each of them having only one peak on gas chromatography. All the quinones were completely converted to the related dihydroxyl compounds under silylation; cis- and trans-4,5-diols were stable in both columns; cis- and trans-7,8-diols and trans-9,10-diol were rather unstable, especially in Dexsil-300 columns. Both 7,8-diols showed 3 peaks: the original chemical (30%); 7-OH-BP (60%); and 8-OH-BP (10%). trans-9,10-Diol yielded the original chemical (64%), 9-OH-BP (32%), and 10-OH-BP (4%) on Dexsil-300 columns. Tetraols were stable, but the 4,5-oxide was completely converted to 4-OH-BP (54%) and 5-OH-BP (46%) under silylation.

Diol-epoxide 2 was converted to certain breakdown products under silylation. The chromatographic patterns of the products and the mass spectrum of the major product are shown in Chart 1. The major product was identified as 7,8,9-trihydroxy-7,8-dihydro-BP, because 9-OH-BP was detected as a product derived from 9,10-oxide (21). Diol-epoxide 1 was converted to the same major product. Dihydroxyl compounds and tetraols were also detectable in small amounts.

Mass Spectra of BP Derivatives. Fragment ions and the relative intensities of BP derivatives are summarized in Table 1. The fragmentation patterns of BP and the 12 phenols were very simple, as shown by Yagi et al. (24). Each showed only molecular ions. The fragment ions of phenol at m/e 325, [M — CH3]+, and/or m/e 73, Si+(CH3)3, were very weak. Quinones were checked without silylation. The mass spectra of 1,6-, 3,6-, 6,12-, and 7,10-quinones showed base peaks at m/e 282 which are molecular ions. Other fragment ions appeared at m/e 254 and/or 226, due to ions of [M — CO]+ and [M — 2CO]+, respectively. Base peaks of 4,5- and 11,12-quinones appeared at m/e 254. Their molecular ions at m/e 282 were also intense.

The base peaks of 1,6-, 3,6-, 6,12-, and 7,10-dihydroxy-BP’s were due to molecular ions at m/e 428. The mass spectrum of 4,5-dihydroxy-BP showed a base peak at m/e 428 and 3 fragment ions at m/e 355, 340, and 73. The base peak of 11,12-dihydroxy-BP was due to the molecular ion, and 2 other peaks appeared at m/e 340 and 73.

The mass spectra of diols showed several fragment ions. The dominant fragment ions obtained from cis-4,5-diol were a molecular ion at m/e 430, [M — CH3]+ at m/e 415, [M — TMSOH]+ at m/e 340, C20H27+ at m/e 252, (CH3)2Si(OSi(CH3)3 at m/e 147 (base peak), and Si+(CH3)3 at m/e 73. The mass spectra of trans-4,5-diol, cis-11,12-diol, and trans-11,12-diol showed fragmentation patterns very similar to that of cis-4,5-diol. The mass spectra of cis- and trans-7,8-diols were almost identical. Their fragment ions appeared at m/e 430, 340, 327, 191, 147, and 73. The fragment ion at m/e 191 was due to (TMSO)2CH+. The mass spectrum of trans-9,10-diol showed a fragmentation pattern very similar to that of the 7,8-diols except for the base peak at m/e 73. The intensities of the fragment ions varied depending on the ionization voltage.

The mass fragmentation of tetraols was rather simple. The mass spectra of 4 tetraols showed 3 to 4 fragment ions of molecular ion at m/e 608, [M — TMSOH]+ at m/e 518, [M — TMSOCHCHOTMS]+ at m/e 404, and TMSOC+HOTMS at m/e 191.

Ratios of Retention Time. Among the samples, trans-4,5-diols gave the shortest retention time (less than 1 min), and tetraol cis-1a gave the longest retention time (17 min). Their retention times varied when the analytical conditions were changed. Therefore, a ratio of retention time was adopted to...
Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention time</th>
<th>Fragmentation</th>
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<tr>
<td></td>
<td>Dexsil-300</td>
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<tr>
<td>BP</td>
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<td>2-OH-BP</td>
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<tr>
<td>3-OH-BP</td>
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<td>4-OH-BP</td>
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<td>11,12-Quinone</td>
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<td>trans-4,5-Diol</td>
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<tr>
<td>trans-11,12-Diol</td>
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<td>0.14</td>
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</table>

- All hydroxyl compounds were trimethylsilylated.
- Ratios were calculated from elution of internal standards of benz(a)anthracene (0.00) and dibenz(a,h)anthracene (1.00).
- Numbers in parentheses, relative intensity (percentage).
- Unstable.
- Eluted faster than benz(a)anthracene.

obtain constant values. The ratio can be provided from the retention times of the 2 internal standards of benz(a)anthracene (0.00) and dibenz(a,h)anthracene (1.00). Errors of the retention time were within 2% with this method. The ratios of retention time tended to become lower with aging of the columns; therefore they were discarded when the ratios of retention time shifted more than 2%. The ratios obtained are shown in Table 1.

Separation of BP Derivatives. The 12 phenols formed 5 peaks in the OV-1 column (1 m), as shown in Chart 2a. 8- and 11-OH-BPs were separated completely; the others were separated incompletely. 1-, 3-, 6-, 7-, and 9-OH-BP's, which had been identified directly or indirectly in biological materials (13, 14, 16), were applied to a Dexsil-300 column (2 m). 6-, 7-, and 9-OH-BP's were separated, but 1- and 3-OH-BP's were fused, as shown in Chart 2b. All quinones were completely converted to related dihydroxyl compounds under silylation. 11,12-, 4,5-, 3,6-, and 1,6-dihydroxy-BP's were completely separated in the OV-1 column (1 m), but 6,12- and 7,10-isomers were eluted together. Their fragmentogram is shown in Chart 3.

DISCUSSION

Polycyclic aromatic hydrocarbons have been isolated by GC-MS in the course of research on air pollution (9) and tobacco smoke (10), but their hydroxyl metabolites have not yet been demonstrated in tissues as far as we know. Stomning and Bresnik (18) separated the silylated 11,12-diol of 3-MC by gas chromatography for the assay of epoxide hydrase, and Betten court et al. (1) used 'K-region' diols of benz(a)anthracene, BP, and 3-MC to GC-MS for the same purpose. One of the present authors was successful in separating several hydroxyl...
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Chart 2. Mass fragmentograms of silylated phenols. In a, all phenols were applied to an OV-1 column (1 m). In b, the phenols that had been demonstrated in biological materials were applied to a Dexsil-300 column (2 m). BA, benz(a)anthracene; DBA, dibenz(a,h)anthracene.

Chart 3. Mass fragmentogram on OV-1 column (1 m) of silylated dihydroxyl compounds. BA, benz(a)anthracene; DBA, dibenz(a,h)anthracene.

Chart 4. Mass fragmentogram on OV-1 column (1 m) of silylated diols. BA, benz(a)anthracene; DBA, dibenz(a,h)anthracene.

Chart 5. Mass fragmentogram on OV-1 column (1 m) of silylated stereoisomeric 7,8,9,10-tetraols. BA, benz(a)anthracene; DBA, dibenz(a,h)anthracene.

Identification of BP Metabolites by GC-MS

a 5-,6-4-, 1-,2-10-,12-7-3-,9-

Identification of BP Metabolites by GC-MS

isomers of the same group could not be separated from each other by mass fragmentography alone because they have almost the same fragmentation patterns. Therefore, they should be separated by gas chromatography only. Unfortunately, some isomers have the same or very close retention times, and the separation of 2 isomers was incomplete when the ratios of retention time differed less than 5 to 7% on 1-m columns. We checked the retention time by different columns, e.g., OV-17, OV-101, SE-30, Thermol-3, and Dexsil-410, but the retention times were very close to those on OV-1 and Dexsil-300, and the order of elution on all the columns used showed no change among the isomers of the same group.

Not all diols could be separated completely, but the separation was sufficient for practical purposes, because those detected in biological materials were only trans-diols (17) and all trans-diols studied were separated completely.

Diol-epoxides have been reported to be unstable (23) and readily converted to tetraols in water (8, 25). In the present experiment, diol-epoxides 1 and 2 were converted to 7,8,9-trihydroxy-7,8-dihydro-BP under silylation. Therefore, it is impossible to identify diol-epoxides themselves under the present analytical conditions. However, the presence of stereoisomeric 7,8,9,10-tetraols and/or 7,8,9-trihydroxy-7,8-dihydro-BP strongly suggest the presence of diol-epoxides in the test materials.

Despite some instabilities and incomplete separations of certain compounds, GC-MS was verified to be an effective system for the rapid separation and accurate identification of BP-related hydroxyl compounds.

ACKNOWLEDGMENTS

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