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PRELIMINARY COMMUNICATIONS

SEPARATION OF TEN BENZO(A)PYRENE PHENOLS BY RECYCLE HIGH PRESSURE LIQUID CHROMATOGRAPHY AND IDENTIFICATION OF FOUR PHENOLS AS METABOLITES

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Benzo(a)pyrene (BP) is a common environmental pollutant and carcinogen of the polycyclic aromatic hydrocarbon (PAH) type (1). The microsomal enzyme complex aryl hydrocarbon hydroxylase (AHH) and metabolically related enzymes convert PAH's to arene oxides, phenols, dihydrodiols, quinones and water-soluble conjugates(2-6). The AHH complex functions in detoxification (7,8) and activation of PAH's to reactive intermediates (9,6) which are toxic and form covalent linkage with DNA in vitro (9). Inhibition of AHH by 7,8-benzoflavone results in both reduced tumorigenicity and covalent binding of DMBA to DNA (9). To understand the pathways of detoxification and activation of PAH's it is necessary to isolate and identify their metabolites. We have shown that reverse phase high-pressure liquid chromatography (HPLC) is a sensitive and rapid technique for the isolation of BP metabolites (2,10). We found that phenols formed metabolically migrated in two peaks which contained largely 3-OH-BP and 9-OH-BP. With a similar system the 12 synthetic phenols of BP were also not separable but eluted in two peaks (11). We now report new systems which resolved all ten of the synthetic phenols available to us. With this technique we have determined the positions at which BP is metabolically converted to phenols. We have found that BP is converted to 3-OH-BP (3,5), 9-OH-BP (5) as previously reported and newly isolated 1-OH-BP and 7-OH-BP.

Methods: The BP phenols were prepared by the following methods: 3-OH-BP (12); 6-OH-BP (13); 7-OH-BP (14); 8-OH-BP and 9-OH-BP (15), 10-OH-BP Engel et al (in preparation) and 1-OH-BP (16). The 4-OH-BP and 5-OH-BP isomers were made as diacetates (17) and converted to phenols. The 12-OH-BP was synthesized from 12-methylbenz(a)anthracene via bromination with n-bromosuccinimide, reaction with NaCN to 12-cyanomethylbenz(a)anthracene, hydrolysis and cyclization with HF. The synthetic or metabolically formed phenols were dissolved in dioxane: N-hexane 40:60. One to two μ l (containing less than 1 μg) solution was injected at approximate concentrations giving similar absorbance at 254 Mu. The chromato-

graphy was by a modification of the recycle system of Henry et al (18) using a HPLC fitted with two Zorbax-sil columns (25 cm), connected to a six-port valve assembly and arranged for flow thru either two columns in series or thru a 8 µl UV flow cell between columns. The solvent mixture was hexane:dioxane (9:1) containing 40 ul formic acid per 200 ml solvent. The columns were isocratically run at ambient temperature and the pressure was 1800 psi. The peaks were collected and the retention times and UV spectra compared to authentic standards. Metabolites formed from BP by rat liver microsomes were prepared as previously described (5). The metabolites were then separated by standard HPLC (2,10) into phenol, dihydrodiol and quinone regions. The phenols were collected and injected into the recycle HPLC system described here.

Results: Fig 1 shows the elution profile of ten synthetic benzo(a)pyrene phenols after a total of one to five passes without interruption thru two columns by means of a recycling valve (18). The 5-OH is eluted first followed by a peak containing the 4-OH and 6-OH. The latter cannot be recycled because their passage thru the second column is completed before the last peak (8-OH, 9-OH) is eluted from the first column. The 6-OH and 4-OH however can be separated by the ODS-permaphase system previously described (2,10). The first peak to be recycled contains 12-OH and 10-OH with 7-OH as a shoulder. It is followed by two peaks containing 3-OH and 1-OH and the 8-OH and 9-OH. The first recycled peak appearing after passage thru three columns contains 12-OH and 10-OH as a shoulder. These are further recycled. The 7-OH peak is collected and the following peak containing 3-OH and 1-OH is recycled. The next two peaks containing 9-OH and 8-OH respectively are collected. The 12-OH and 10-OH and the 1-OH and 3-OH are recycled and the peaks collected. Better resolution of the latter can be obtained by further recycling.

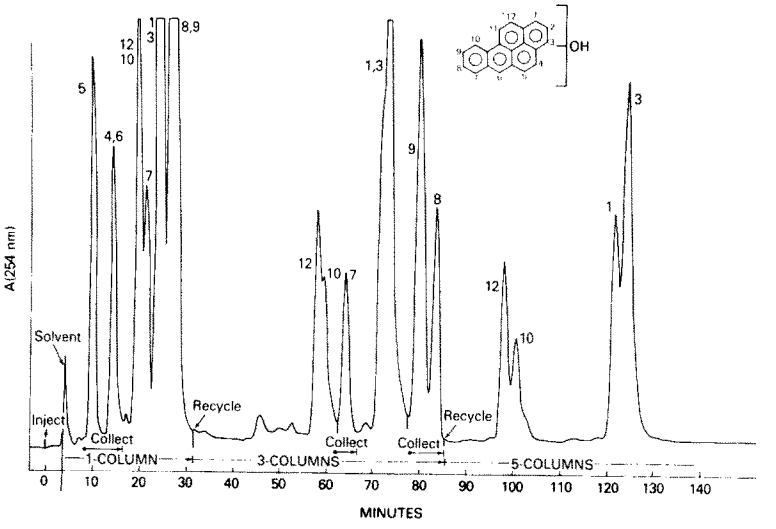

Fig 1. Recycle HPLC of Synthetic Benzo(a)pyrene Phenols

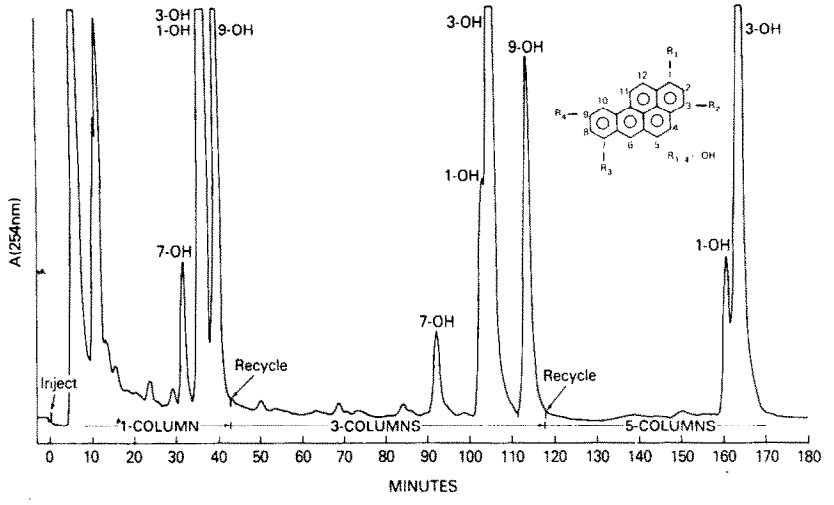

Fig 2. Recycle HPLC of Benzo(a)pyrene Phenol Metabolites

Fig 2 shows the separation of phenols formed metabolically. The first two peaks are non BP related U.V. absorbing material. Three metabolite peaks are observed after passage thru a single column. Four distinct peaks are resolved after passage thru five columns. Each peak was isolated and characterized by its relative mobility compared to the standard and comparison of its U.V. spectra with the authentic synthetic phenol. The identified phenols are 3-OH-BP (2,3) and 9-OH-BP (5) also previously reported, and two metabolites, the 1-OH and 7-OH which are newly isolated and identified by the HPLC system described.

In an early report (19) a BP metabolite was partially characterized and identified as 1-OH-BP. A synthetic standard was not available and the characterization is not directly comparable to the present study.

The present report establishes methodology for the separation of benzo(a)-pyrene phenols and demonstrates that four of these phenols are BP metabolites. They are clearly the major phenol metabolites and further quantitative studies will determine whether they constitute the total BP phenol formation. The method we describe can be used to determine the primary catalytic sites of the various forms of the mixed function oxygenases. Highly purified P-450's convert BP to phenol peaks with different fluorescent spectra (20). The new method will help determine the site preference of different mixed function oxygenases for BP hydroxylation and their role in carcinogen activation and detoxification.

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