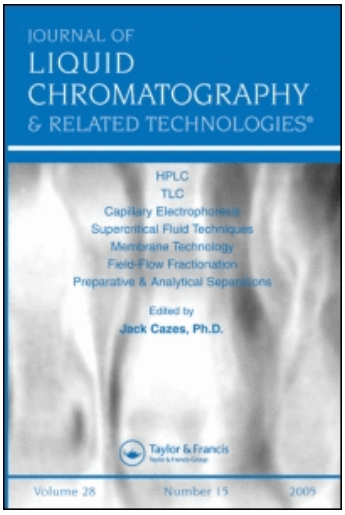

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ISOLATION OF STABLE MUTAGENIC PHOTODECOMPOSITION PRODUCTS OF BENZO(a)PYRENE BY THIN-LAYER CHROMATOGRAPHY[1,2]

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are widespread in the atmosphere, the soil, waterways and oceans, as well as in the food chain. Major sources of PAH include emissions from transportation systems, heat and power generating plants, refuse burning and industrial processes (1,2,3). A significant number of these PAH are known to be carcinogenic (2,4,5). Epidemiological studies indicate that the environment is a significant factor in the incidence of human cancer, although many of the specific causal agents remain to be identified. One widely studied PAH is benzo(a)pyrene (BaP), which is a carcinogen, and one of the most common PAH contaminants of the environment

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(6). Occupational exposure to BaP has been documented (4). Mailath and Morik (7) studied the effect of sunlight and dark-room storage on BaP and reported that six and eight compounds, respectively, were observed when BaP was exposed to sunlight or left in the dark. There have been several reports of the degradation of BaP adsorbed on soot or smoke particles (8,9,10).

Despite the fact that a number of thin-layer chromatographic (TLC) methods have been described for the separation of polycyclic aromatic hydrocarbons, the results must be treated with caution. As early as 1964, Inscoe (11) reported that some PAH are unstable when applied and developed on silica gel plates. Rao and Vohra (12) noted a rapid fading of benzo(a)pyrene (BaP) spots on silica gel plates, and concluded that any *in situ* evaluation was impossible. Seifert (13) noted this, and suggested that impregnation of the plate with 5% paraffin wax could reduce BaP degradation.

We have examined BaP, one of the most-studied carcinogenic environmental contaminants, in solution and on TLC plates (silica gel and cellulose), under artificial and natural UV irradiation. Since this compound is found in the environment, its exposure to sunlight may result in the formation of stable photodecomposition products that may represent an unevaluated human hazard. Preliminary evidence indicates that formation of stable products can occur under ambient conditions during exposure to sunlight or artifical UV light. The potential for human carcinogenic risk from this phenomena merits investigation.

Maisin and de Johnghe observed that light accelerates the production of skin tumors by BaP (14). In another skin painting study, it was found that 31% of the group treated with BaP showed tumors whereas 63% of the group treated with BaP plus irradiation showed tumors (15). Irradiation of certain kinds is also carcinogenic and might be a promoting agent. Therefore, the activity of photolysis products might be a factor.

The objectives of this study were to examine the effects of UV radiation on the stability of BaP in solution and on TLC plates, to isolate the stable photodecomposition products and to test for their possible mutagenic activity.

EXPERIMENTAL

BaP was obtained from Aldrich Chemical Co., Inc. (Metuchen, NJ, USA). Methanol, ethyl acetate and benzene were glass distilled from Burdick and Jackson (Muskegon, MI, USA). TLC silica gel plates were from E. Merck (Elmsford, NY, USA). Elution of the spots from the plates were carried out by using an automatic elution system, the Eluchrom by Camag (New Berlin, WI, USA). A 15 amp Xenon lamp, Zenarch from Nems and Clarke (Silver Spring, MD, USA) was used for the photodecomposition of BaP. Fluorescence measurements were made on MPE2 Spectrofluorometer (Perkin-Elmer).

Mutation tests were carried out essentially as described by Ames et al. (16). Variations were the use of 0.2 ml of the tester strain, 20 ml of VBE AGAR and 75 µl of Aroclor 1254-stimulated rat microsomes per plate. All testing was done with the plate incorporation assay using either methanol (MeOH), or undiluted dimethylsulfoxide (Me_2SO) as the solvent. Plates were incubated for 48 hrs. at 37°C and the revertant colonies were counted using a hand-held tally.

Solutions of BaP, 334 µg/ml, were streaked on silica gel plates, allowed to dry, exposed to sunlight or UV radiation for one hour, and then developed in benzene:ethyl acetate (9:1). Regions from the developed plates were eluted and tested for mutagenicity.

RESULTS AND DISCUSSION

Fluorescence measurements indicated that the BaP solutions examined were stable, but turned yellow when subjected to excessive UV radiation. When solutions of BaP were spotted on silica gel, alumina, cellulose and reversed-phase TLC plates and dried under nitrogen, followed by development and elution, no loss due to decomposition was observed. However, when the such solutions were spotted on the plates and allowed to dry in the hood in a stream of air, several developed spots were observed. Moreover, similarities in the BaP results were obtained when plates spotted with BaP were exposed to sunlight (Table 1).

TABLE 1

R_f VALUES OF PHOTO-DECOMPOSITION PRODUCTS OF BaP ON SILICA GEL PLATES AFTER 15 MINUTES EXPOSURE TO SUNLIGHT

Compound #	R_f	Fluorescence Color under Long UV
1	0-0.1	Greenish
2	0.24	Faint
3	0.37	Faint
4	0.42	Red (1,6-BaP Quinone)
5	0.48	Yellow (3,6-BaP Quinone)
6	0.57	Yellow
7	0.72	Faint yellow
8	0.82	Faint red
9	0.95	Blue (BaP)

Two of these products were eluted off the plate and were characterized as the 1,6- and 3,6-BaP quinones by chromatographic and spectroscopic techniques.

The two regions from the BaP plate (R_f, 0.0 - 0.1, R_f, 0.4 - 0.5) were found to be direct acting mutagens (Table 2). When a BaP methanol solution, 334 µg/ml, was subjected to UV light for three hours and subsequently tested for mutagenicity, the results were again positive, with and without metabolic activation (Table 3). BaP was mutagenic for both the base pair substitution strain TA 100 and the frameshift strain TA 1538. Further experimentation is planned to discern the identity of the product(s) exhibiting mutagenic activity. These preliminary data suggest that the photodecomposition product(s) may be more highly mutagenic than the parent hydrocarbon. It should be noted that

TABLE 2

The Number of Revertant Colonies Using Tester Strain TA 1538 With and Without Added Rat Liver Microsomes for Unexposed BaP and Eluted TLC Plate Regions of Irradiated BaP*

Test Material	Concentration	no S_9	S_9 added
Cells only	--	10	26
MeOH	--	11	27
Unexposed BaP	50 µg	12	225
Eluted TLC Region 1 (R_f, 0.4 - 0.5)	50 µg	81	171
Cells only	--	12	23
Me_2SO	undiluted	18	22
Unexposed BaP	50 µg	24	330
Eluted TLC Region (R_f, 0.0 - 0.1)	50 µg	43	32

* Mutagenic results are the average of two runs.

TABLE 3

The Number of Revertant Colonies Using Tester Strain TA 100 With and Without Added Rat Liver Microsomes for BaP Before and After Irradiation for Three Hours With UV Light*

Test Material	Concentration	no S_9	S_9 Added
Cells only	--	167	154
MeOH	--	116	124
BaP	33.4 µg	154	1008
BaP Irradiated in MeOH	33.4 µg	537	2371

* Mutagenic results are the average of two runs.

the two identified photodecomposition products of BaP (1,6- and 3,6-quinones) cannot account for the high mutagenicity observed.

Hence, an understanding of the photo-oxidation processes of PAH components, as well as the biological assessment and structural characterization of their stable photodecomposition products on coated TLC plates and on standard dust samples, may lead to the identification of a previously insufficiently appreciated area of environmental carcinogens to which humans are exposed.

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