Journal of Chromatography, 291 (1984) 231–239
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,601

DEMONSTRATION OF MICROSOMAL OXYGENATION OF THE BENZO RING OF 6-NITROBENZO[*a*]PYRENE BY THIN-LAYER CHROMATOGRAPHY

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(Received January 25th, 1984)

SUMMARY

To explain the biological activity of 6-nitrobenzo[*a*]pyrene (6-nitroB*a*P), male Sprague-Dawley rats were induced with 3-methylcholanthrene. Liver microsomes were incubated with magnesium chloride, an NADPH generating system and 6-nitroB*a*P in acetone. The mixture was chilled under oxygen-free argon gas and protein was precipitated with an equal volume of cold methanol containing triethylamine. Protein was further precipitated with zinc and sodium sulfate and centrifuged. Both the sediment and the supernatant were extracted with benzene and ethyl acetate. The organic extract was washed with water, 2% sodium hydroxide solution, water and then dried with anhydrous sodium sulfate. Solvents were removed and the residue was chromatographed on silica gel plates with hexane containing increasing amounts of benzene. The UV and mass spectra of products were examined. Liver microsomal metabolites of 6-nitroB*a*P consisted of 7,8- and 9,10-dihydrodiols and also benzo[*a*]pyrene (B*a*P) and B*a*P-quinones. *cis*-Forms of 6-nitroB*a*P-7,8- and -9,10-dihydrodiols were synthesized.

INTRODUCTION

Nitro-substituted polycyclic aromatic hydrocarbons (PAHs) are found in fly ash, diesel emissions, photocopier fluids, cigarette smoke and other environmental samples[1]. 6-Nitrobenzo[*a*]pyrene (6-nitroB*a*P) is mutagenic in microbial test systems[2] and some nitro-PAHs are tumorigenic in animals[3].

Benzo[*a*]pyrene (B*a*P), the parent compound of 6-nitroB*a*P, produces oxygenated derivatives during metabolic activation[4]. To explain the biological activity of 6-nitroB*a*P, we examined whether such oxygenated derivatives are also formed from 6-nitroB*a*P during metabolism[2,5].

EXPERIMENTAL

6-NitroB*a*P was prepared according to Fieser and Hershberg[6]. The brownish yellow nitration product of B*a*P (Aldrich, Milwaukee, WI, U.S.A.) was percolated

TABLE I

R_F VALUES

Compound	*Solvent 1: hexane benzene (3:1)*	*Solvent 2: hexane–benzene (1:1)*	*Solvent 3: hexane–benzene (2:3)*	*Solvent 4: benzene*	*Solvent 5: benzene chloroform (3:1)*	*Solvent 6: benzene–dichloro-methane (97:3)*	*Solvent 7: chloroform–ethyl acetate (3:1)*	*Solvent 8: Methanol*
B*a*P (known)	0.43	–	–	–	–	0.93	–	0.86
6-NitroB*a*P	0.34	–	–	–	–	0.85	–	0.64
DinitroB*a*P in crude 6-nitroB*a*P	–	–	–	–	–	–	–	0.57
Synthetic								
6-NitroB*a*P-7,8-dihydrodiol (*cis*) (light blue)-O-diacetate	0.1	0.25, 0.25	–	–	0.62	–	0.98	–
6-NitroB*a*P-9,10-dihydrodiol (*cis*) (faint blue)	–	0.27	–	–	0.65	–	–	–
Metabolic	0.1	0.14, 0.15	–	–	0.58	–	0.90	–
6-NitroB*a*P-7,8-dihydrodiol (*trans*) (bright blue)	0.17, 0.20	0.18, 0.21, 0.22	0.29, 0.35	0.64, 0.64	0.59	–	0.91	–
O-diacetate	–	–	–	–	0.58	–	–	–
6-NitroB*a*P-9,10-dihydrodiol (*trans*) (violet)-O-diacetate	0, 0.08	0.09, 0.09, 0.11	0.15, 0.18	0.30, 0.30	0.44	–	–	–
Unknown hydrocarbon (violet blue)	–	–	–	–	0.45	–	–	–
	0.70, 0.71	0.76	0.87	–	–	–	–	–

TABLE II

UV SPECTRA OF SYNTHETIC AND METABOLIC PRODUCTS

Compound	*Wavelength (nm) (absorbance)*
6-Nitro*Ba*P	262 (0.428), 268 (0.495), 275 (0.306), 277 (0.301), 283 (0.268), 290 (0.37), 296 (0.29), 303 (0.43), 315 (0.115), 330 (0.035), 357 (0.132), 362 (0.139), 375 (0.199), 382 (0.172), 384 (0.179), 387 (0.178), 392 (0.189), 402 (0.13), 405 (0.14), 410 (0.118)
Synthetic -7,8-dihydrodiol (*cis*)	250 (0.828), 255 (0.848), 263 (0.605), 267 (0.658), 274 (0.38), 278 (0.405), 282 (0.368), 289 (0.632), 294 (0.4), 301 (0.795), 313 (0.05), 330 (0.04), 338 (0.068), 344 (0.07), 355 (0.18), 360 (0.162), 373 (0.346), 381 (0.221), 387 (0.27), 389 (0.28), 393 (0.378), 402 (0.068), 407 (0.11), 410 (0.075)
-O-diacetate	278 (0.198), 282 (0.178), 289 (0.281), 295 (0.182), 302 (0.359), 310 (0.092), 330 (0.029), 338 (0.04), 355 (0.099), 360 (0.09), 373 (0.163), 382 (0.112), 387 (0.132), 394 (0.178), 403 (0.045), 407 (0.061), 410 (0.049)
-9,10-dihydrodiol	273 (0.23), 280 (0.218), 290 (0.182), 299 (0.101), 303 (0.091), 310 (0.05), 345 (0.03), 365 (0.046), 374 (0.049), 380 (0.039), 395 (0.038), 400 (0.033), 410 (0.03)
Metabolic-7,8-dihydrodiol (*trans*?)	268 (0.75), 274 (0.704), 278 (0.72), 286 (0.68), 292 (0.71), 298 (0.55), 304 (0.65), 325 (0.199), 349 (0.215), 360 (0.228), 369 (0.265), 380 (0.23), 388 (0.248), 405 (0.25), 408 (0.26)
O-diacetate	278 (0.178), 283 (0.161), 289 (0.187), 296 (0.135), 302 (0.196), 313 (0.05), 325 (0.038), 333 (0.04), 340 (0.035), 354 (0.07), 360 (0.067), 373 (0.092), 380 (0.071), 387 (0.078), 393 (0.098), 402 (0.041), 407 (0.048), 410 (0.042)
Unknown (identified as B*a*P)	276 (0.48), 280 (0.426), 287 (0.67), 293 (0.46), 299 (0.82), 310 (0.115), 320 (0.082), 334 (0.102), 339 (0.101), 350 (0.21), 356 (0.18), 368 (0.352), 376 (0.211), 380 (0.29), 385 (0.351), 387 (0.4), 400 (0.06), 403 (0.045), 410 (0.05)

in benzene through a silica gel column to collect a yellowish eluate. The residue from the eluate was repeatedly chromatographed in 100-mg quantities on 1-mm thick 20 × 20 cm silica gel plates (J. T. Baker, Phillipsburg, NJ, U.S.A.) with hexane–benzene (1:1) (Table I), until a yellow product, without any orange coloration (which would connote the presence of B*a*P quinones), was obtained. The yellow 6-nitroB*a*P used for metabolism experiments had an m.p. of 251–252°C and UV and mass spectral properties, as shown in Tables II and III, respectively. The 6-nitroB*a*P mass spectrum contained no peak at *m/e* 252 due to B*a*P (Table III).

Thin-layer chromatographic separation of oxygenated B*a*P derivatives and their UV spectra have been described previously[7]. 6-NitroB*a*P derivatives were identified by comparing their properties with those of the B*a*P products. UV spectra were taken with a Cary 15 spectrophotometer and mass spectra by an AEI MS9 instrument (70 eV) with a direct inlet probe at 220°C. All biochemicals were from Sigma (St. Louis, MO, U.S.A.).

Male Sprague–Dawley rats (80–100 g) were injected intraperitoneally with 3-methylcholanthrene (MC) at 25 mg/kg body weight for three consecutive days. Liver microsomes were prepared according to Nebert and Gelboin[8]. Protein was determined by the Lowry method[9] and the cytochrome P-450 content according to Omura and Sato[10].

TABLE III

MASS SPECTRAL CHARACTERISTICS OF PRODUCTS

Compound	*m/e*	*Composition*	*Observed mass*	*Intensity*	*Groups (molecules) ejected*
6-NitroB*a*P* (synthetic)	297	$C_{20}H_{11}NO_2$	297.0782	100	none
	281	$C_{20}H_{11}NO$	281.0834	2.66	O
	280	$C_{20}H_{10}NO$	280.0758	1.88	OH
	269	$C_{19}H_{11}NO$	269.0834	5.19	CO
	267	$C_{20}H_{11}O$	267.0831	42.55	NO
	264	$C_{20}H_{10}N$	264.0804	1.63	OH, O
	253	$C_{19}H_{11}N$	253.0907	2.23	CO, O
	251	$C_{20}H_{11}$	251.0843	57.99	NO_2
	250	$C_{20}H_{10}$	250.0781	85.66	OH, NO
	241	$C_{18}H_{11}N$	241.0868	6.33	CO, CO
	239	$C_{19}H_{11}$	239.0.839	49.41	CO, NO
Synthetic 6-nitroB*a*P-7,8-dihydrodiol** (*cis*)	331	$C_{20}H_{13}NO_4$ (parent)	none	–	–
	268	$C_{20}H_{12}O$	268.0878	64.48	OH, NO_2
	267	$C_{20}H_{11}O$	267.0820	15.07	OH, OH, NO
	239	$C_{19}H_{11}$	239.0838	32.76	NO, CO, OH, OH
	238	$C_{19}H_{10}$	238.0758	4.10	CHOH, OH, NO_2
-O-diacetate	415	$C_{24}H_{17}NO_6$	none	–	–
	310	$C_{22}H_{14}O_2$	310.0981	15.25	$O \cdot CO \cdot CH_3$, NO_2
	268	$C_{20}H_{12}O$	268.0889	100	CH_2CO, $O \cdot CO \cdot CH_3$, NO_2
	267	$C_{20}H_{11}O$	267.0818	17.81	CH_3CO, $O \cdot CO \cdot CH_3$, NO_2
	239	$C_{19}H_{11}$	239.0865	46.19	$O \cdot CO \cdot CH_3$, $O \cdot CO \cdot CH_3$, CO, NO
		Formed by two H transfer $[CH_3 \cdot C(OH)(OH)]^+$			
		$C_2H_4O_2$ $[CH_3 \cdot CO \cdot OH]^{+\cdot}$	60.0216	0.92	
-9,10-dihydrodiol (*cis*)***	331	$C_{20}H_{13}NO_4$ (parent)	none	–	–
	268	$C_{20}H_{12}O$	268.0896	20.66	OH, NO_2
	267	$C_{20}H_{11}O$	267.0816	3.86	OH, OH, NO
	239	$C_{19}H_{11}$	239.0867	10.46	NO, CO, OH, OH
	238	$C_{19}H_{10}$	238.0769	0.79	CHOH, OH, NO_2
Metabolic 7,8-dihydrodiol (*trans*)§	331	$C_{20}H_{13}NO_4$ (parent)	none	–	–
	268	$C_{20}H_{12}O$	268.0878	28.22	OH, NO_2
	267	$C_{20}H_{11}O$	267.0789	6.64	OH, OH, NO
	239	$C_{19}H_{11}$	239.0870	25	NO, CO, OH, OH
	238	$C_{19}H_{10}$	238.0796	3.08	CHOH, OH, NO_2
	225	$C_{18}H_9$	225.0686	14.65	CHOH, CHOH, NO_2
	73	$C_3H_5O_2$ $[(CH(CHOH)\,CHOH)]^+$	73.0297	1.00	
-O-diacetate	310	$C_{22}H_{14}O_2$	310.0966	5.66	$O \cdot CO \cdot CH_3$, NO_2
	268	$C_{20}H_{12}O$	268.0880	40.87	CH_2CO, $O \cdot CO \cdot CH_3$, NO_2
	267	$C_{20}H_{11}O$	267.0824	7.90	CH_3CO, $O \cdot CO \cdot CH_3$, NO_2

TABLE III *(continued)*

Compound	*m/e*	*Composition*	*Observed mass*	*Intensity*	*Groups (molecules) ejected*
	239	$C_{19}H_{11}$	239.0857	17.97	O · CO · CH_3, O · CO · CH_3, NO, CO
	61	$C_2H_5O_2$	61.0289	5.86	
	60	$C_2H_4O_2$	60.0221	1.21	
-9,10-dihydrodiol O-diacetate§	239	$C_{19}H_{11}$	239.0839	1.05	O · CO · CH_3, CO, NO
		Concentration of sample was small			
Metabolic hydrocarbon (Tentatively identified as B*a*P)	252	$C_{20}H_{12}$	252.0943	60.47	
	251	$C_{20}H_{11}$	251.0840	5.93	
		Both had doubly charged ions suggesting aromatic characters.			
	126	$C_{10}H_6$	126.0464	8.07	
			125.5419	and 1.15	
		Fragment due to C_2H_2 loss was seen at			
	226	$C_{18}H_{10}$	226.0769	2.50	

* Double charged ions were present. There were ions due to acetylene (C_2H_2) loss.

** Unlike metabolic trans-7,8-dihydrodiol there was no peak at M/E 225. Double charged ions and nitrogen containing fragments of lower masses were present.

*** Nitrogen-containing fragments of low intensity were present. Fragmentation patterns of synthetic 7,8- and 9,10-dihydrodiols were different from each other.

§ Fragments due to acetylene (C_2H_2) loss were present. Pathways differed slightly for metabolic and synthetic dihydrodiols.

The final incubation volume for the metabolism studies was 83.3 ml. To 80 ml of 50 m*M* Tris buffer, pH 7.5, containing 162 mg microsomal protein (82.42 nmole, cytochrome P-450) 3.2 mmole $MgCl_2 \cdot 6H_2O$ was added and the incubation was conducted at 37°C for 4 min. Glucose-6-phosphate (0.46 mmole), NADP (0.04 mmole) and glucose-6-phosphate dehydrogenase (375 Sigma units) were added and the incubation was continued for another 5 min. NADPH generation was detected by examining the absorbance at 340 nm. 6-NitroB*a*P (12.45 μmole) in 3.3 ml acetone was added and the incubation was continued in air on a shaking bath for an additional 55 min.

The incubation mixture was then bubbled with oxygen-free argon gas while being chilled on ice. Argon gas was used throughout the isolation procedure until the mass spectrum was determined. To the chilled reaction mixture, 83.3 ml precooled methanol containing triethylamine (100 μl used with 1000 ml methanol) was added to protect the dihydrodiols against decomposition by acids on glass surfaces.

To the reaction mixture, 0.5 g $ZnSO_4 \cdot 7H_2O$ and 50 g $Na_2SO_4 \cdot 10H_2O$ were added. The mixture was shaken vigorously to salt out the protein, which was then sedimented with a laboratory centrifuge.

The sedimented protein was extracted with benzene, ethyl acetate and methanol (50 ml each) and the extraction repeated until no more yellow color (due to excess 6-nitroB*a*P) was extracted into the organic solvents. The benzene and ethyl acetate extracts were saved in a 500-ml separatory funnel. The methanol extracts

were added to the aqueous supernatant obtained during the protein centrifugation. Pre-cooled water (480 ml or three times the volume of methanol) was added to dilute the methanol and the resultant mixture was extracted with 100 ml benzene and four times with 100 ml ethyl acetate. During each of these extractions, a large excess of sodium chloride was added to salt out the water-soluble metabolites. The main aqueous layer was saved.

The combined organic extract (*ca.* 450 ml) was then fractionated to separate phenolic and neutral substances by washing twice with 50 ml water to remove salt, then with 50 ml pre-cooled 2% sodium hydroxide solution. (Indicator paper showed the extract to be alkaline.) The UV spectrum of the sodium hydroxide extract was recorded to detect phenols. The organic material was extracted once more with 50 ml 2% sodium hydroxide solution. After taking the UV spectra, the alkaline and aqueous washes were combined with the main aqueous layer above and extracted with 75 ml ethyl acetate to obtain additional yellow material. All organic extracts were combined. The combined organic layer (*ca.* 550 ml) was washed twice with 50 ml water, and dried overnight over 100 g anhydrous sodium sulfate.

The solvent was removed (35°C and 35 mm) and the greenish-yellow residue was chromatographed on a 0.25-mm thick silica gel plate (20 × 20 cm) (Table I). All initial applications of samples to an 18-cm wide streak were with methanol–triethylamine to protect dihydrodiols and then with dichloromethane, benzene, ethyl acetate and again with methanol (five solvents). Nitrogen gas (used to dry the samples) and argon were passed through nylon tubing, instead of tygon tubing, to avoid contamination with substances which interfere with mass spectrometric determinations.

The plate was developed for one hour with hexane–benzene (75:25) (Table I, solvent 1) in argon-filled Shandon tanks. The wet plate was viewed under UV light (both 360 and 254 nm), and the R_F values, the color and fluorescence of nine bands were noted. Each of these bands was extracted with the five solvents mentioned above. The solvents were removed and the residues chromatographed [Table I, solvent 2: hexane–benzene (50:50)]. During the chromatography with solvent 2, the width of the streaking area was selected according to the size of the residue. A number of samples could be applied on the same 20 × 20 cm plate by cutting vertical grooves, end to end, with a razor blade. This side-by-side application of samples permitted comparison of their R_F values and fluorescence after a run. After run 2, bands of unconverted 6-nitroB*a*P (yellow visible and dark under UV) moved toward solvent front. These fractions were combined and the UV spectrum (Table II) taken to estimate recovered 6-nitroB*a*P (absorbance 1 at 392 nm = 20.47 μg/ml dichloromethane solution).

After recombination, only four bands other than 6-nitroB*a*P were obtained and were chromatographed in solvent 3 (hexane–benzene, 40:60). Again bands with the same R_F values and fluorescence were combined. In our work with oxygenated B*a*P derivatives[7] the dihydrodiols were found to have blue fluorescence under UV light. Therefore, in the recombination work, bands with blue fluorescence were particularly checked. After a run in solvent 3 only the blue bands were further chromatographed in solvents 4, 5 and 7 (Table I). After this, the blue fluorescent dihydrodiols were extracted into 1.5–3 ml dichloromethane and the UV spectra were recorded. The samples were concentrated and their mass spectra were determined.

From the UV spectra, a bluish metabolic product (Tables I, II and III) appeared to be 6-nitroB*a*P-7,8-dihydrodiol[7]. A spectral shift from B*a*P-7,8-dihydrodiol was observed due to the nitro group. For purposes of comparison, the *cis*-forms of these substances were synthesized. Dichloromethane (100 ml), taken in a 250-ml erlenmeyer flask fitted with a 24/40 ground glass stopper, was cooled in ice and 5.9 mg 6-nitroB*a*P was added and swirled to dissolve. Then 120 mg *m*-chloroperoxybenzoic acid (MCPBA, Aldrich, 80–90%) was added, swirled to dissolve and left in the cold room for 96 h. The yellowish, light orange reaction mixture was treated twice with 50 ml saturated sodium sulfite solution to destroy excess MCPBA and then twice with 50 ml saturated sodium bicarbonate solution to remove some of the *m*-chlorobenzoic acid. This treatment also hydrolyzes epoxides to dihydrodiols. The organic layer was dried over 50 g anhydrous sodium sulfate, filtered and the solvent was removed. The yellowish residue was chromatographed (Table I, solvents 1, 2, 5 and 7). Repeated runs in solvent 2 were necessary to separate 9,10- from 6-nitroB*a*P-7,8-dihydrodiol. R_F values of 6-nitroB*a*P-9,10-dihydrodiol were smaller than those of the 7,8-derivative (Table I).

Both the synthetic and metabolic 7,8- and 9,10-dihydrodiols were also converted to O-diacetyl derivatives (Tables I, II and III). Anhydrous pyridine and sodium-treated acetic anhydride were chilled separately in ice. Equal volumes of the two reagents were mixed, chilled again and approximately 2 ml of the chilled acetylating reagent was added to a sample of dried dihydrodiol (a few μg) and mixed. This mixture was allowed to stand at room temperature for at least 24 h. It was then decomposed with five volumes ice water and extracted three times with an equal volume dichloromethane. The dichloromethane extract (*ca.* 12 ml) was washed with water, 5% acetic acid solution, water, dried over anhydrous sodium sulfate, and the solvent was removed. The residues were chromatographed (Table I) and the UV and mass spectra of these O-diacetyl derivatives recorded (Tables II and III).

RESULTS AND DISCUSSION

Results showed that the UV spectrum of a 2% sodium hydroxide wash of the organic extract of an incubation mixture did not contain any phenolic substances[2,5]. It remains to be determined whether the phenols were formed and subsequently decomposed to quinones during the duration of the incubation (55 min). Due to the presence of excess 6-nitroB*a*P in the crude mixture, nine bands were obtained when the neutral incubation residue was chromatographed in solvent 1 (Table I). Each of these bands on running in solvent 2 separated into unconverted 6-nitroB*a*P, which appeared yellow under visible light and dark under UV light, with R_F value 0.4–0.6. The combined 6-nitroB*a*P bands showed a recovery of 2.05 mg (3.7 mg incubated). In solvent 2, other substances were observed. In ascending order, there was: a reddish band R_F 0, quinones; a light blue band, R_F 0.09, 6-nitro-B*a*P-9,10-dihydrodiol; a blue band, R_F 0.18, 6-nitro-B*a*P-7,8-dihydrodiol; and a visibly yellow band (or dark band under UV), R_F value 0.4–0.6 (different plates), 6-nitroB*a*P. A bright blue fluorescent substance was also noted with an R_F value 0.76, greater than that of 6-nitro-B*a*P, The chromatographic properties (Table I) of this substance suggested it to be a hydrocarbon. The mass spectrum (Table III) of our starting 6-nitroB*a*P did not contain a peak at *m/e* 252 due to BaP. The UV[7] and mass spectra (Table III) of this hydro-

carbon obtained from the incubation suggested this to be B*a*P (0.08 μg per mg protein; 3.7 mg 6-nitroB*a*P incubated). Ono *et al.*[11] have shown that 1-benzyl-1,4-dihydronicotinamide, an analog of NADPH, replaces aliphatic nitro groups by hydrogen. It remains to be established whether NADPH converted 6-nitroB*a*P to B*a*P.

In pure benzene (solvent 4), yellow, red and other colored quinones with smaller R_F values separated out of the dihydrodiols, in particular, 6-nitroB*a*P-7,8-dihydrodiol. We rechromatographed some of these quinones in dichloromethane–benzene (97:3)[7]. From the R_F values they appeared to be B*a*P-1,6-, 3,6- and 6,12-quinones. The mass spectrum of one such quinone had peaks at *m/e* 282, 254 and 226. On the basis of UV determination, 6-nitroB*a*P-7,8-dihydrodiol was the major dihydrodiol, the yield of which was estimated as B*a*P-7,8-dihydrodiol[7] to be around 0.006 μg per mg protein (3.7 mg 6-nitroB*a*P incubated). The yield of 9,10-dihydrodiol was still less. The yield of synthetic 6-nitroB*a*P-7,8-dihydrodiol was approximately 16 μg. In one metabolic experiment, we purified the 6-nitro-B*a*P-7,8-dihydrodiol by running in solvents 1, 2, 5 and 7 (Table I). The UV and mass spectra of this dihydrodiol suggested the blue fluorescing substance to contain B*a*P-6,12-quinone as a contaminant. The UV peaks (absorbance) (Table II) were: 268 (0.75), 278 (0.72), 292 (0.71), 304 (0.65), 350 (0.215), 369 (0.265), 388 (0.248). Mass spectra did not contain any peak for the molecular ion of the dihydrodiol. The mass spectral ions (intensity) [molecules (groups) ejected] (Table III) were: $C_{19}H_{10}O$ (42.77) (CHOH, OH, NO); $C_{20}H_{12}O$ (28.22) (OH, NO_2); $C_{18}H_9$ (14.65) (CHOH, CHOH, NO_2); $C_{20}H_{11}O$ (6.64) (OH, OH, NO). Since B*a*P quinones tenaciously adhere to the 6-nitroB*a*P dihydrodiol, to separate them from 6-nitroB*a*P dihydrodiols we acetylated the latter. The chromatographic properties and UV and mass spectra of O-acetylated metabolic products, together with their synthetic counterparts (Tables I, II and III), suggest that acetylation could be used to both stabilize and purify 6-nitroB*a*P dihydrodiols.

The chromatographic, UV and mass spectral[12] data of products (Tables I, II and III) suggest that rat liver microsomes and a chemical reagent (MCPBA) introduce oxygen into the 7,8 and 9,10-positions of 6-nitroB*a*P. Also produced were B*a*P and B*a*P quinones in the microsome reaction. In a control experiment without the microsomes, some of the quinones, but not the other products, were formed. The intermediate epoxides and (or) secondary metabolites of the dihydrodiols may be responsible for the biological activity of 6-nitroB*a*P.

ACKNOWLEDGEMENT

This work was supported by funds provided by the Nebraska Department of Health through LB-506.

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