

CHROM. 15,862

Note

Thin-layer chromatographic separation and spectra of oxygenated benzo[*a*]pyrene derivatives in dichloromethane

C. R. RAHA

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68105 (U.S.A.)

(Received March 6th, 1983)

Benzo[*a*]pyrene (BaP), a polycyclic aromatic hydrocarbon found in our environment, induces tumors in experimental animals and is implicated in human cancer. BaP requires metabolic activation for carcinogenicity and produces oxygenated derivatives¹. We describe a thin-layer chromatographic (TLC) method as a substitute for expensive high-performance liquid chromatography⁷ and time-consuming paper chromatography methods^{2,3}. This TLC method separates BaP-7,8-dihydrodiol, the precursor of BaP-7,8-dihydrodiol 9,10-epoxide (BPDE), which is the ultimate carcinogen of BaP⁴, from other oxygenated BaP derivatives. We also discuss the use of dichloromethane, which extracts smaller amounts of interfering substances from TLC plates and thereby facilitates mass spectrometric examination of the metabolites.

BaP (Aldrich, Milwaukee, WI, U.S.A.) was crystallized^{5,6}. Oxygenated BaP derivatives (through Dr. David G. Longfellow, NCI Chemical Repository of the IIT Research Institute, Chicago, IL, U.S.A.) were used as received.

Milligram amounts of the substances were dissolved in 10 ml of dichloromethane. BaP-*cis*- and *trans*-4,5-dihydrodiols required 30 ml of warm solvent, while BaP-7,*trans*-8,9-triol called for 100 ml. These solutions were then diluted (Table I) and the UV spectra were recorded between 410 and 250 nm with a Cary 15 UV-VIS spectrophotometer.

Silica gel plates (UV-254, Brinkman Instruments, Westbury, NY, U.S.A.) were used in an ascending direction in Shandon tanks. The synthetic samples were applied either as spots (singles) or as 3-cm wide streaks (mixtures). They were dried (nitrogen) and developed in the respective solvents for at least 1 h (Table II). The fluorescence of wet plates and the R_F values of the substances were noted under both 360-nm and 254-nm UV lamp. BaP-4,5-dihydrodiols do not fluoresce under 360 nm, but rather appear as dark spots under a 254-nm UV-lamp. When the same plate was run in more than one solvent it was dried in a vacuum oven at room temperature for 15 min and then developed in the next solvent.

For recovery, UV absorbance of *trans*-BaP-4,5-dihydrodiol (highly polar) was recorded. The solution was concentrated, applied to a 1-cm area, dried, scraped off, extracted with dichloromethane and concentrated. The spectrum was then recorded to determine recovery.

For mass spectral determination, an AEI MS-9 instrument with a direct inlet probe at 220°C was used.

TABLE I
UV SPECTRA OF OXYGENATED BENZO[*a*]PYRENE DERIVATIVES

<i>Compound (µg/ml dichloromethane with which the spectra were taken)</i>	<i>Wavelength (nm) (absorbance)</i>
Benzo[<i>a</i>]pyrene (BaP) (1.36)	280 (0.11), 287 (0.189), 293 (0.12), 299 (0.26), 310 (0.005), 350 (0.06), 357 (0.05), 367 (0.118), 376 (0.065), 380 (0.1), 387 (0.14), 400 (0.009), 404 (0.02). 11.14 µg/ml at 385 nm.
<i>Phenols</i>	
1-OH-BaP (1.19)	265 (0.175), 269 (0.19), 282 (0.105), 289 (0.158), 294 (0.128), 300 (0.2), 320 (0.015), 350 (0.03), 378 (0.11), 384 (0.1), 388 (0.11), 398 (0.119), 410 (0.035) 10 µg/ml at 398 nm.
2-OH-BaP (1.28)	274 (0.191), 283 (0.16), 289 (0.178), 299 (0.122), 305 (0.138), 325 (0.021), 353 (0.068), 360 (0.061), 371 (0.111), 379 (0.078), 383 (0.09), 391 (0.108), 405 (0.02), 410 (0.03) 11.85 µg/ml at 391 nm.
3-OH-BaP (1.0)	265 (0.13), 270 (0.149), 278 (0.059), 280 (0.062), 293 (0.105), 300 (0.078), 307 (0.145), 320 (0.008), 363 (0.065), 370 (0.05), 380 (0.1), 390 (0.041), 397 (0.07), 410 (0.021) 14.28 µg/ml at 397 nm.
4-OH-BaP (1.05)	269 (0.295), 280 (0.138), 290 (0.152), 295 (0.142), 302 (0.18), 310 (0.038), 336 (0.035), 345 (0.04), 355 (0.08), 360 (0.08), 373 (0.118), 380 (0.1), 387 (0.105), 395 (0.102), 405 (0.05), 410 (0.055) 10.29 µg/ml at 395 nm.
5-OH-BaP (1.0)	267 (0.195), 275 (0.12), 277 (0.125), 284 (0.105), 291 (0.165), 296 (0.125), 304 (0.185), 315 (0.02), 355 (0.058), 367 (0.07), 376 (0.091), 390 (0.078), 396 (0.088), 403 (0.04), 407 (0.092) 11.36 µg/ml at 396 nm.
6-OH-BaP (2.36)	268 (0.295), 276 (0.181), 282 (0.208), 285 (0.199), 293 (0.318), 297 (0.24), 305 (0.41), 315 (0.048), 370 (0.12), 378 (0.138), 388 (0.185), 403 (0.155), 410 (0.171) 12.75 µg/ml at 388 nm.
7-OH-BaP (2.0)	263 (0.422), 269 (0.51), 285 (0.258), 293 (0.43), 297 (0.331), 304 (0.632), 320 (0.04), 358 (0.155), 364 (0.14), 376 (0.288), 387 (0.172), 398 (0.299), 410 (0.07) 6.68 µg/ml at 398 nm.
8-OH-BaP (1.57)	160 (0.372), 265 (0.368), 270 (0.388), 274 (0.386), 278 (0.412), 288 (0.308), 291 (0.321), 303 (0.268), 306 (0.271), 331 (0.045), 336 (0.051), 342 (0.049), 354 (0.129), 359 (0.112), 372 (0.22), 378 (0.141), 391 (0.22), 403 (0.02) 7.13 µg/ml at 391 nm.
9-OH-BaP (1.7)	263 (0.33), 270 (0.422), 276 (0.315), 288 (0.438), 298 (0.239), 304 (0.305), 315 (0.028), 361 (0.172), 367 (0.129), 379 (0.279), 388 (0.1), 393 (0.149), 405 (0.03) 6.09 µg/ml at 379 nm.
10-OH-BaP (2.2)	266 (0.285), 277 (0.175), 280 (0.179), 285 (0.161), 292 (0.295), 297 (0.23), 304 (0.46), 315 (0.045), 335 (0.014), 360 (0.096), 367 (0.088), 379 (0.179), 388 (0.11), 400 (0.181), 405 (0.1) 12.29 µg/ml at 379 nm.

TABLE I (continued)

<i>Compound (µg/ml dichloromethane with which the spectra were taken)</i>	<i>Wavelength (nm) (absorbance)</i>
11-OH-BaP (2.3)	260 (0.385), 269 (0.61), 282 (0.23), 292 (0.32), 297 (0.286), 303 (0.355), 315 (0.05), 330 (0.021), 364 (0.16), 368 (0.138), 377 (0.223), 380 (0.219), 383 (0.242), 388 (0.148), 393 (0.2), 405 (0.045) 9.50 µg/ml at 383 nm.
12-OH-BaP (2.82)	262 (0.351), 269 (0.399), 275 (0.335), 289 (0.462), 293 (0.461), 298 (0.528), 310 (0.1), 320 (0.032), 360 (0.132), 365 (0.13), 377 (0.258), 387 (0.178), 399 (0.265), 405 (0.135) 10.93 µg/ml at 377 nm.
<i>Diones</i>	
BaP-4,5-dione (2.73)	260 (0.451), 267 (0.658), 271 (0.662), 275 (0.8), 286 (0.208), 295 (0.048), 305 (0.05), 312 (0.072), 315 (0.078), 320 (0.096), 327 (0.125), 331 (0.119), 337 (0.14), 348 (0.108), 352 (0.1), 355 (0.05) 19.5 µg/ml at 337 nm.
BaP-1,6-dione (13.6)	250 (0.891), 253 (0.93), 257 (0.19), 260 (0.93), 287 (0.192), 290 (0.191), 295 (0.15), 302 (0.162), 317 (0.091), 410 (0.455) 83.95 µg/ml at 302 nm.
BaP-6,12-dione (3.6)	275 (0.188), 282 (0.249), 286 (0.241), 292 (0.281), 298 (0.19), 304 (0.25), 316 (0.045), 352 (0.13), 360 (0.119), 369 (0.159), 385 (0.059) 22.64 µg/ml at 369 nm.
BaP-3,6-dione (3.68)	250 (0.26), 255 (0.24), 263 (0.211), 266 (0.215), 286 (0.089), 292 (0.091), 298 (0.05), 304 (0.059), 309 (0.058), 317 (0.082), 324 (0.072), 342 (0.105), 370 (0.069) 35.04 µg/ml at 345 nm.
BaP-7,8-dione (3.27)	320 (0.222), 338 (0.43), 344 (0.402), 352 (0.515), 365 (0.145) 6.34 µg/ml at 352 nm.
BaP-11,12-dione (3.48)	258 (0.378), 276 (0.81), 294 (0.1), 308 (0.071), 350 (0.16), 385 (0.038) 21.75 µg/ml at 350 nm.
<i>Dihydrodiols and triol</i>	
<i>cis</i> -BaP-4,5-dihydrodiol (1.83)	260 (0.241), 261 (0.39), 268 (0.38), 275 (0.615), 285 (0.09), 297 (0.061), 300 (0.071), 306 (0.053), 313 (0.071), 320 (0.042), 326 (0.069), 335 (0.01) 26.52 µg/ml at 326 nm.
<i>trans</i> -9,10- (3.12)	260 (0.155), 272 (0.34), 275 (0.348), 282 (0.455), 287 (0.371), 288 (0.378), 297 (0.152), 302 (0.212), 313 (0.058), 318 (0.075), 323 (0.061), 332 (0.151), 338 (0.07), 348 (0.282), 355 (0.035) 11.06 µg/ml at 348 nm.
<i>trans</i> -4,5- (2.79)	260 (0.455), 262 (0.48), 264 (0.47), 271 (0.741), 290 (0.076), 296 (0.091), 303 (0.079), 308 (0.092), 315 (0.051), 322 (0.09), 330 (0.012) 31 µg/ml at 322 nm.
<i>cis</i> -7,8- (3.71)	260 (0.465), 269 (0.118), 270 (0.113), 277 (0.111), 284 (0.241), 289 (0.161), 296 (0.325), 309 (0.031), 335 (0.181), 340 (0.165), 351 (0.43), 359 (0.266), 368 (0.577), 380 (0.085), 390 (0.022), 395 (0.035), 400 (0.018) 5.37 µg/ml at 368 nm.
7,8,9-Tetrahydrotriol (ca. 4)	265 (0.099), 269 (0.195), 274 (0.1), 281 (0.36), 288 (0.019), 310 (0.035), 316 (0.08), 321 (0.065), 331 (0.21), 337 (0.099), 347 (0.32) Approx. 12.5 µg/ml at 347 nm.

TABLE II
R_F VALUES

Compound	Solvent 1: hexane- benzene (3:1)	Solvent 2: dichloro- methane	Solvent 3: dichloromethane- benzene (97:3)	Solvent 4: ethyl acetate	Solvent 5: dichloromethane- ethanol (9:1)	Solvent 6: methanol-THF (19:1)
BaP	0.5, 0.52	0.87	0.88			
1-OH-BaP	0, 0	0.34, 0.36, 0.44	0.54			
2-OH	0	0.33				
3-OH	0, 0	0.36, 0.36	0.32, 0.44, 0.44, 0.53	0.76, 0.76		
4-OH	0	0.49				
5-OH	0	0.40				
6-OH	0	0.51, 0.54	0.68			
7-OH	0, 0	0.39	0.36, 0.49, 0.50	0.77		
8-OH	0	0.25				
9-OH	0, 0	0.27	0.24, 0.34, 0.36	0.76		
10-OH	0	0.54				
11-OH	0	0.54				
12-OH	0	0.40				
<i>cis</i> -4,5-Diol	0, 0	0	0, 0.01, 0.02	0.61, 0.69, 0.72, 0.73	0.77, 0.82	0.82
<i>trans</i> -9,10-Diol	0, 0	0, 0	0.006, 0.007, 0.01	0.42, 0.44, 0.49, 0.51	0.57, 0.63	0.83
7,8,9-Triol	0	0		0.08	0.36	0.80
<i>trans</i> -4,5-Diol	0	0	0, 0.015, 0.01, 0.02	0.71, 0.73	0.71, 0.73	0.82
<i>cis</i> -7,8-Diol	0, 0	0, 0	0, 0.015, 0.01, 0.02	0.48, 0.51, 0.53, 0.57	0.66, 0.72	0.80
BaP-4,5-quinone	0	0.23		0.85		
BaP-1,6-quinone	0, 0	0.06, 0.12	0.06, 0.06, 0.08, 0.09	0.70, 0.77, 0.78		
BaP-6,12-quinone	0, 0	0.10, 0.24	0.12, 0.14, 0.15, 0.16	0.72, 0.83, 0.88		
BaP-3,6-quinone	0, 0	0.04, 0.10	0.04, 0.04, 0.05, 0.06	0.65, 0.76		
BaP-7,8-quinone	0	0.20		0.87		
BaP-11,12-quinone	0	0.32		0.87		

Table II shows that a sequence of dichloromethane-based solvents separates BaP, BaP-phenols, the quinones and the dihydrodiols. In solvent 1 (hexane-benzene, 3:1), BaP has an R_F of 0.5. The BaP-phenols, quinones and the dihydrodiols have zero R_F . For large-scale metabolite mixtures, solvent 1 is suitable to remove unchanged BaP on silica gel columns. In solvent 2 (dichloromethane) BaP is at the solvent front with R_F 0.87, the blue fluorescent phenols have R_F values of 0.25-0.54 and the colored quinone—yellow (4,5-, 1,6-, 6-12-), red (3,6-), violet (7,8-) or pink (11,12-)—have R_F values smaller than the phenols. The dihydrodiols have zero R_F in dichloromethane.

A mixture of 1-OH-, 3-OH-, and 6-OH-BaP, BaP-1,6-, 3,6- and 6,12-diones, and BP-*trans*-4,5-, -*trans*-9,10-, and -*cis*-7,8-dihydrodiol was run in dichloromethane. 1-OH- and 3-OH-BaP did not separate (R_F 0.33). 6-OH-BP separated out at R_F 0.54, and the three quinones formed two spots: (1) red, R_F 0.05 and (2) yellow, R_F 0.15. When this plate was developed in dichloromethane-benzene (97:3), all three quinones separated out. The R_F values were as follows: quinones, 1,6-, 0.09; -3,6-, 0.06; -6,12-, 0.16. 1-OH- and 3-OH-BaP still remained mixed. The dihydrodiol moved very little (Table II). In a mixture of 3-OH-, 7-OH- and 9-OH-BaP, the quinones and the dihydrodiols and the phenols separated well in dichloromethane-benzene (97:3). R_F values: 3-OH-, 0.44; 7-OH-, 0.52; and 9-OH-, 0.33. Quinone R_F values were as mentioned earlier.

The dihydrodiols were best separated with ethyl acetate. There was further resolution, including separation of *cis*- and *trans*-4,5-dihydrodiols in dichloromethane-ethanol (9:1). In the latter solvent, even the triol moved. BaP-7,8-dihydrodiol, the precursor of BPDE, separated out from the other dihydrodiols. R_F values: ethyl acetate, *cis*-4,5, and *trans*-4,5-dihydrodiol as a mixture, 0.72; *trans*-9,10-, 0.51, and *cis*-7,8-, 0.57. Values for the same plate after dichloromethane-ethanol (9:1) were *cis*-4,5-, 0.78; *trans*-4,5-, 0.71; *trans*-9,10-, 0.57, and *cis*-7,8-, 0.66. In methanol-tetrahydrofuran (solvent 6) the diols and the triol had large R_F values. We examined the separation of a mixture (as if from a metabolic experiment) of 185 μg BaP with 1.8 μg each of 3-OH-, 7-OH-, 9-OH-, 1,6-, 3,6-, and 6,12-diones and *cis*-4,5-, *trans*-9,10-, and *cis*-7,8-dihydrodiols. In dichloromethane-benzene (97:3), the phenols were a mixture at R_F 0.53, the colored quinones at 0.37, the bluish dihydrodiols at 0.06, and BaP at R_F 0.86. The phenols, quinones and the dihydrodiols were extracted with ethyl acetate, dichloromethane and methanol. The solutions were concentrated and separated, as described above. A sequence of solvents 3, 4 and 5 (Table II) separates a mixture of oxygenated BaP derivatives.

We recovered 51% BaP-*trans*-4,5-dihydrodiol with dichloromethane extraction. Either BaP or 3-OH-BaP, after a chromatographic run, was extracted with dichloromethane and the mass spectrum determined without interference: BaP ($\text{C}_{20}\text{H}_{12}$, 252.0931) and 3-OH-BaP ($\text{C}_{20}\text{H}_{12}\text{O}$, 268.0892). In Table I, UV spectra (maxima-minima) are given to allow reconstruction of the spectra. The last line indicates $\mu\text{g}/\text{ml}$ (factor) that produces an absorbance of 1 at the wavelength mentioned. Concentration (total μg) = ml \times absorbance \times factor.

ACKNOWLEDGEMENT

This work was supported by funds provided by the Nebraska Department of Health through legislative bill 506.

REFERENCES

- 1 H. V. Gelboin, *Physiol. Rev.*, 60 (1980) 1107.
- 2 C. R. Raha, *Indian J. Biochem. Biophys.*, 9 (1972) 105.
- 3 C. R. Raha, C. H. Gallagher, P. Shubik and S. Peratt, *J. Nat. Cancer Inst.*, 57 (1976) 33.
- 4 P. Sims, P. L. Grover, A. Swaisland, K. Pal and A. Hewer, *Nature (London)*, 252 (1974) 326.
- 5 C. R. Raha, C. H. Gallagher and P. Shubik, *Proc. Soc. Exptl. Biol. Med.*, 143 (1973) 531.
- 6 C. R. Raha, L. K. Keefer and J. Loo, *J. Chem. Eng. Data*, 18 (1973) 332.
- 7 J. K. Selkirk, R. G. Croy, P. P. Roller and H. V. Gelboin, *Cancer Res.*, 34 (1974) 3474.