Biochemical Pharmacology, Vol. 16, pp. 613–618. Pergamon Press Ltd. 1967. Printed in Great Britain

THE METABOLISM OF BENZO[a]PYRENE BY RAT-LIVER HOMOGENATES

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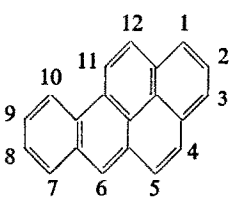
(*Received* 6 *December* 1966)

Abstract—In addition to 3-hydroxybenzo[a]pyrene and benzo[a]pyrene-1,6 and 3,6-quinones, benzo[a]pyrene was converted by rat-liver homogenates into two previously unrecognized dihydrodihydroxy compounds. One of these yielded 1-hydroxybenzo[a]pyrene with acid and appeared to be 1,2-dihydro-1,2-dihydroxybenzo[a]pyrene. The second metabolite, which was tentatively identified as 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene, yielded a phenol with acid whose u.v. absorption spectrum was identical with that of the so-called F_1 metabolite of benzo[a]pyrene. The phenol was tentatively identified as 8-hydroxybenzo[a]pyrene.

ALTHOUGH there have been many investigations on the metabolism of benzo[a]pyrene (I) in the animal body, the metabolic pathways involved are still far from clear. Earlier work, summarized by Williams,[1] indicated that the hydrocarbon was hydroxylated in the 1-, 3- and 6-positions, whilst more recently it has been shown[2] that 1,6 and 3,6-dihydroxy compounds are also formed, together with a number of unidentified metabolites. In the work described below the metabolism of benzo[a]pyrene by rat-liver homogenates has been investigated using methods previously described.[3]



(I)

EXPERIMENTAL

Thin-layer chromatograms were prepared by coating glass plates (20 × 20 cm) with silica gel of 0·25 mm thickness. Chromatograms were developed for 15 cm with either (a) light petroleum (b.p. 80–100°)-benzene, (b) benzene or (c) benzene–ethanol (19:1, v/v). The chromatograms were examined in u.v. light whilst still wet both before and after exposure to ammonia. Two-dimensional chromatograms were developed with solvent (c) in the first direction, sprayed with conc. HCl and heated to 100° for 10 min and developed in the second direction with solvent (b). In this way phenols arising from the decomposition of acid-labile dihydrodihydroxy compounds were detected. Ultraviolet absorption spectra of material obtained by eluting

with ethanol bands or spots of silica gel removed from thin-layer chromatograms were measured on a Unicam SP 800 recording spectrophotometer.

Benzo[a]pyrene was obtained from Koch-Light Ltd. and 3-methoxybenzo[a]-pyrene was the gift of Dr. R. Schoental. 6-Acetoxy and 6-hydroxybenzo[a]pyrene and 7-hydroxybenzo[a]pyrene were prepared by published methods[4, 5] and a mixture of benzo[a]pyrene-1,6- and 3,6-quinones was obtained from the oxidation of the hydrocarbon with CrO_3.[6]

Benzo[a]pyrene (100 mg) was oxidized with OsO_4 as described by Cook and Schoental[7] and a portion of the product was chromatographed on a thin-layer chromatogram, which was developed with solvent (c). Two bands were detected by their fluorescence in u.v. light: the u.v. spectra of the two products were not alike (see Table 2), that of the slower moving compound resembling that of chrysene and that of the faster moving compound resembling that of benz[a]anthracene. The slower moving compound, which was the major product of the oxidation, is therefore probably *cis*-4,5-dihydro-4,5-dihydroxybenzo[a]pyrene, which was previously isolated[7] from this oxidation mixture whilst the faster moving compound is *cis*-11,12-dihydro-11,12-dihydroxybenzo[a]pyrene. Both compounds yielded phenols and other unidentified products on the two-dimensional chromatograms described above.

Young male rats of the Chester Beatty Strain (body wt. approx. 180 g) were used. In some experiments the animals were pretreated with 3-methylcholanthrene (5 mg) in arachis oil (0·5 ml), administered by intraperitoneal injection 40 hr before the animals were killed: this treatment increased the yields, but did not affect the nature, of the products. In each experiment, the livers from eight rats were pooled, homogenized and incubated with benzo[a]pyrene (10 mg) in the presence of co-factors as previously described.[3] The products were isolated from the incubation mixtures with ethyl acetate and applied to the base lines of four thin-layer chromatograms which were developed with solvent (b). The chromatograms were each divided into three fractions, using the fluorescence in u.v. light as a guide, to yield a fast-moving fraction containing mainly unchanged hydrocarbon, an intermediate fraction containing phenols and quinones and the material left at the base lines which contained dihydrodihydroxy compounds. The fractions were removed from the chromatograms, like fractions being combined, and the absorbed material was eluted from the silica gel with ether and the residues remaining on evaporation of the solvent were chromatographed using one fresh plate for each fraction. The fastest-moving fraction was chromatographed in solvent (a), the intermediate fraction in solvent (b) and the third fraction in solvent (c). Bands of metabolites were detected in u.v. light as before and were examined as described below.

RESULTS

The only material found in the fastest-moving fraction was benzo[a]pyrene itself, which was identified by its u.v. absorption spectrum and by its chromatographic properties.

The intermediate fraction contained three major products together with traces of unidentified material. Of the three products identified, one was undoubtedly 3-hydroxybenzo[a]pyrene. It exhibited a characteristic change in fluorescence when chromatograms were examined in u.v. light and exposed to ammonia (see Table 1)

TABLE 1. PROPERTIES OF COMPOUNDS RELATED TO BENZO[a]PYRENE ON THIN-LAYER CHROMATOGRAMS

Compound	R_f in solvent			Fluorescence in u.v. light		Remarks
	(a)	(b)	(c)	Immediate	After exposure to ammonia	
1. Benzo[a]pyrene	0·45	0·95	0·95	Violet	Violet	
2. 3-Hydroxybenzol[a] pyrene	0·00	0·37	0·55	Violet	Green	
3. 6-Hydroxybenzo[a]-pyrene	0·00	0·55	0·65	Blue	Blue–green	
4. 7-Hydroxybenzo[a]-pyrene	0·00	0·43	0·62	Blue	Orange	
5. Benzo[a]pyrene 1,6-quinone	0·00	0·20	0·55	Yellow	Yellow	Yellow on untreated chromatograms
6. Benzo[a]pyrene 3,6-quinone	0·00	0·10	0·45	Orange	Orange	Red on untreated chromatograms
7. Metabolite, probably 1,2-dihydro-1,2-dihydrobenzo[a]-pyrene	0·00	0·00	0·21	Violet	Violet	
8. Phenol, probably 1-hydroxybenzo[a]-pyrene	0·00	0·45	0·62	Violet	Pink	Formed from Compound 7 on two-dimensional chromatograms
9. Metabolite, probably 7,8-dihydro-7,8-dihydroxybenzol[a]-pyrene	0·00	0·00	0·15	Violet	Violet	
10. Phenol, probably 8-hydroxybenzo[a]-pyrene	0·00	0·35	0·53	Violet	Bright green	Formed from Compound 9 on two-dimensional chromatograms
11. Compound, probably 4,5-dihydro-4,5 dihydroxy-benzo[a]pyrene	0·00	0·00	0·25	Violet	Violet	
12. Phenol, probably 5-hydroxybenzo[a]-pyrene	0·00	0·42	0·61	Violet	Yellow	Formed from Compound 11 on two-dimensional chromatograms
13. Compound, probably 11,12-dihydro-11, 12 dihydroxybenzo[a]-pyrene	0·00	0·00	0·28	Violet	Violet	
14. Phenol, probably 12-hydroxybenzo[a]-pyrene	0·00	0·40	0·60	Violet	Yellow	Formed from Compound 13 on two-dimensional chromatograms

One and two dimensional chromatograms were developed as described in the text. Some variation in R_f values occurred between different chromatograms: the values quoted are typical.

and its u.v. spectrum (Table 2) was identical with that of the authentic phenol described by Conney *et al.*[8] The methyl ether, obtained by the methylation of a portion of the eluted metabolite with diazomethane in ether and chromatographing the product in solvent (b) was undistinguished on chromatograms from authentic 3-methoxybenzo[a]pyrene and the u.v. spectra of the two ethers were identical. The phenol has been isolated as a metabolite of benzo[a]pyrene in whole animals.[9]

TABLE 2. ULTRAVIOLET ABSORPTION SPECTRA OF COMPOUNDS RELATED TO BENZO[a]PYRENE

Compound	Absorption maxima in mμ
Metabolite, probably 3-hydroxybenzo-[a]pyrene*	258 (3·2), 268 (3·1), 293 (2·1), 307 (2·3), 344 (0·53), 362 (1·2), 380 (1·9), 400 (1·1) and 424 (1·0)
Methyl ether of above	257 (2·3), 264 (2·4), 293 (1·9), 306 (2·3), 345 (0·51), 362 (0·9), 380 (1·5), 399 (1·0) and 422 (1·0)
Synthetic 3-methoxybenzo[a]pyrene	257 (2·4), 264 (2·3), 293 (1·9), 306 (2·3), 345 (0·4), 362 (0·9), 380 (1·5), 399 (1·0) and 421 (1·0)
Metabolite, probably 1,2-dihydro-1,2-dihydroxybenzol[a]pyrene	261 (0·94), 272 (0·31), 283 (0·47), 293 (0·53), 317 (0·15), 333 (0·34), 347 (0·79) and 367 (1·0)
Phenol, probably 1-hydroxybenzo[a]-pyrene*	258 (11·8), 267 (14·0), 286 (9·3), 297 (12·2), 355 (3·2), 373 (6·3), 396 (7·9) and 412 (1·0)
Metabolite, probably 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene	268 (1·2), 278 (1·6), 284 (1·2), 298 (0·73), 314 (0·27), 328 (0·51) and 344 (1·0)
Phenol, probably 8-hydroxybenzo[a]-pyrene (F_1)*	267 (3·8), 287 (3·5), 301 (2·3), 344 (0·34), 360 (1·4), 380 (2·1), 394 (1·3) and 412 (1·0)
Product, probably 4,5-dihydro-4,5- di-hydroxybenzo[a]pyrene	263 (123), 273 (182), 297 (20), 309 (21), 323 (20), 346 (1·5) and 364 (1·0)
Product, probably 11,12-dihydro-11,-12-dihydroxybenzo[a]pyrene	258 (9·0), 262 (9·0), 273 (12), 284 (10), 293 (11), 323 (1·9), 340 (1·5) and 358 (1·0)

* For comparative values see Conney *et al.*[8]
The spectra were measured as described in the text. The figures in parenthesis are absorption intensities related to those of the longest wavelength maxima.

The other two products were indistinguishable on thin-layer chromatograms from benzo[a]pyrene 1,6- and 3,6-quinones, which gave characteristic yellow and red spots respectively on untreated chromatograms. The red quinone appeared to be the major product.

Apart from 3-hydroxybenzo[a]pyrene, no other phenols were detected in this fraction. In particular, a search for the presence of compounds with the expected properties of 1-, 6- and 7-hydroxybenzo[a]pyrene was unsuccessful. When, however, the 6-hydroxy compound itself was incubated with liver-homogenate and the products examined as described above, benzo[a]pyrene 1,6- and 3,6-quinones were the only recognizable products, so that it is likely that the 6-hydroxy compound and possibly the 1,6- and 3,6-dihydroxy compounds were present initially in the mixture of metabolites of benzo[a]pyrene. 3-Hydroxybenzo[a]pyrene was itself readily oxidized in air to the 3,6-quinone.

The fraction containing dihydrodihydroxy compounds yielded two products. The characteristic u.v. absorption spectrum of the faster-moving product is recorded in Table 2. On two-dimensional thin-layer chromatograms it yielded a phenol, the u.v. spectrum of which was identical with that of 1-hydroxybenzo[a]pyrene recorded by Conney *et al.*[8] It seems likely, therefore, that the metabolite itself is 1,2-dihydro-1,2-dihydroxybenzo[a]pyrene.

The second product was present in much greater amount than the first and possessed a different u.v. spectrum (see Table 2). On two-dimensional thin-layer chromatograms it yielded a phenol whose chromatographic properties were similar to, but not identical with, those of 3-hydroxybenzo[a]pyrene (see Table 1). The u.v. spectrum of the new phenol was identical with that of the so-called F_1 metabolite of Wiegert and Mottram[10] as described by Conney *et al.*[8] Possible structures for the metabolite and the phenol are discussed below.

A search was made in this fraction for the presence of dihydrodihydroxy compounds arising from hydroxylation on the 4,5- and 11,12-bonds of benzo[a]pyrene (the *K* regions). Although the synthetic products should have the *cis*-configuration, whereas the metabolites might be expected to have the *trans*-configuration, experience has shown that pairs of isomers of similar compounds derived from other aromatic hydrocarbons have identical or very similar properties on thin-layer chromatograms. No evidence for the presence of benzo[a]pyrene metabolites hydroxylated on the *K* regions was found.

DISCUSSION

The results reported here on the metabolism of benzo[a]pyrene differ in a number of respects from those reported earlier: some of these, however, involved experiments with whole animals. 1-Hydroxybenzo[a]pyrene was reported as a metabolite in mouse skin by Tarbell *et al.*[11] and in homogenates of the livers of young rats by Conney *et al.*:[8] Kotin *et al.* were unable to detect the phenol in whole-body experiments in rats. However, Conney *et al.*[8] chromatographed their products on alumina, which may have had the effect of converting the 1,2-dihydrodihydroxy compound into the 1-hydroxy compound.

6-Hydroxybenzo[a]pyrene has been identified as a metabolite of the hydrocarbon by Casu *et al.*,[12] by Pihar and Spaleny[13] and by Falk *et al.*[2] and 1,6- and 3,6-dihydroxybenzo[a]pyrene are also metabolites.[2, 8] A reason for the absence of these products in the work now described has already been indicated.

Falk *et al.*[2] have adduced evidence for the presence of 4,5-dihydro-4,5-dihydroxybenzo[a]pyrene and its conjugates in rat-bile after the administration of benzo[a]pyrene. It is difficult, however, to reconcile the spectra of their products with that of the synthetic *cis*-4,5-dihydrodihydroxy compound described in Table 2. In the metabolism of aromatic hydrocarbons by rat-liver homogenate, there appears to be no relationship between the ability to form dihydrodihydroxy compounds on the *K* region and carcinogenic activity. Thus compounds of this type have not been detected in benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene[14] metabolism, but are metabolites of benz[a]anthracene and dibenz[a,h]anthracene[15] and of 7- and 12-methylbenz[a]anthracene (P. Sims, unpublished observations).

There have been many attempts in the past to identify the so-called F_1 metabolite. The results of the work now described suggest that it is an artifact arising from the decomposition of a dihydrodihydroxy compound. Although dihydrodihydroxy compounds are themselves stable under physiological conditions, their conjugates especially the sulphuric esters decompose either spontaneously or under mild conditions.[16] Thus, 9,10-dihydro-9-hydroxy-10-phenanthryl sulphate readily yields 9-phenanthrol.[17] The structures of the phenol and the metabolite are still uncertain, but it

has been demonstrated that the phenol is not the 1-, 3-, 6- or 7-hydroxy compound, and it is not either of the phenols (presumably the 4- and 11-hydroxy compounds) produced when the dihydrodihydroxy compounds found at the *K* regions are decomposed with acid. The metabolite itself is neither of these dihydrodihydroxy compounds nor is it the 1,2-dihydrodihydroxy compound. Since F_1 is not 3-hydroxybenzo[a]pyrene, it is unlikely that the metabolite from which F_1 is derived is the 2,3-dihydrodihydroxy compound. It is suggested, therefore, that the metabolite is 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene (or, less likely, the 9,10-derivative) and that the derived phenol, F_1, is 8-hydroxybenzo[a]pyrene. If benzo[a]pyrene is regarded as a derivative of benz[a]anthracene, then the 7,8-bond of the former corresponds with the 8,9-bond of the latter and it has already been shown,[14, 15] that hydroxylation of this bond to yield dihydrodihydroxy compounds is involved in the metabolism of benz[a]anthracene and its derivatives: the dihydrodihydroxy compounds yield mainly 9-hydroxy derivatives with acid.

Acknowledgements—I thank Professor E. Boyland for his interest and Mrs. S. Judd and Mr. A. Hewer for technical assistance. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the Public Health Service Research Grant No. CA-03188-09 from the National Cancer Institute, U.S. Public Health Service.

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