The Metabolism of 7- and 12-Methylbenz[*a*]anthracene and their Derivatives

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1. 7- and 12-Methylbenz[*a*]anthracene were converted by rat-liver homogenates into the corresponding hydroxymethyl derivatives, products that are probably the 8,9-dihydro-8,9-dihydroxy and the 5,6-dihydro-5,6-dihydroxy derivatives, and a number of phenolic products. 2. Both hydrocarbons were converted into glutathione conjugates; that from 7-methylbenz[*a*]anthracene was also formed, together with 5,6-dihydro-5,6-dihydroxy- and 5-hydroxy-benz[*a*]anthracene, from 5,6-epoxy-5,6-dihydro-7-methylbenz[*a*]anthracene. 3. 7- and 12-Hydroxymethylbenz[*a*]anthracene were converted into products that are probably 8,9-dihydro-8,9-dihydroxy derivatives, and into phenols. 4. The preparation of a number of derivatives of the hydrocarbons is described. 5. The oxidation of the hydrocarbons with lead tetra-acetate was investigated.

It has been shown (Boyland & Sims, 1965*a*, 1967) that 7,12-dimethylbenz[*a*]anthracene is converted by rat-liver homogenates into products that include the 7- and 12-hydroxymethyl derivatives of the hydrocarbon and 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[*a*]anthracene. Dihydrodihydroxy derivatives and glutathione conjugates were not detected. The investigations described in the present paper were carried out to determine if the metabolic reactions of the dimethyl compound were typical of other methylbenz[*a*]anthracenes.

EXPERIMENTAL

Melting points are uncorrected.

Absorption spectra

These were measured either on a Perkin-Elmer model 137 or on a Unicam SP.800 recording spectrophotometer. Solutions of products separated on paper or thin-layer chromatograms were obtained by eluting, with methanol, spots cut from paper chromatograms, or, with ethanol, bands removed from thin-layer chromatograms. Absorption spectra are recorded in Table 1.

Chromatography

Paper and thin-layer chromatography were carried out and the products were detected as described by Sims (1966). The solvents used for paper chromatography were (1) butan-1-ol-propan-1-ol-2*N*-NH₃ (2:1:1, by vol.) or (2) butan-1-ol-acetic acid-water (2:1:1, by vol.), and those used for thin-layer chromatography were (a) light petroleum (b.p. 80–100°)-benzene (19:1, v/v), (b) benzene and (c) benzene-ethanol (19:1, v/v). Thin-layer chromatograms

were examined in u.v. light while still wet, both before and after exposure to NH₃. Two-dimensional thin-layer chromatograms used in the investigation of dihydrodihydroxy compounds were developed in solvent (c) for 10 cm., sprayed with conc. HCl, heated at 100° for 10 min. and developed in the second direction with solvent (b).

MATERIALS

*Preparation of 7-methylbenz[*a*]anthracene derivatives.* Roman numerals refer to Scheme 1. 7-Methylbenz[*a*]anthracene (I) and 12-methylbenz[*a*]anthracene (m.p. 136–137° and 135–136° respectively) were prepared by the methods of Wood & Fieser (1940) and of Newman & Gaertner (1950) respectively. 7-Acetoxyethylbenz[*a*]anthracene and 7-hydroxymethylbenz[*a*]anthracene (IV), prepared via 7-chloromethylbenz[*a*]anthracene by the method of Badger & Cook (1940), had m.p. 148° and 171–172° respectively. Benz[*a*]anthracene-7-carboxaldehyde was prepared from benz[*a*]anthracene and *N*-methylformanilide by the method of Badger & Cook (1940). It was oxidized with KMnO₄ to benz[*a*]anthracene-7-carboxylic acid as described by Badger & Cook (1940).

*Oxidation of 7- and 12-methylbenz[*a*]anthracene with lead tetra-acetate.* 7-Methylbenz[*a*]anthracene (1g.) and lead tetra-acetate (2g.), in acetic acid (100 ml.), were heated on the steam bath for 30 min. The mixture was poured into water and the gummy product was chromatographed on silica gel (200–300 mesh; Koch-Light Laboratories Ltd., Colnbrook, Bucks.) in benzene-light petroleum (b.p. 80–100°) (1:1, v/v). Two pale yellow bands were eluted separately and the residues obtained on removal of the solvent recrystallized from ethanol. The material from the faster-moving fraction yielded 7-acetoxyethylbenz[*a*]anthracene in pale yellow needles, m.p. and mixed m.p. 149° (Found: C, 84.35; H, 5.2. Calc. for C₂₁H₁₆O₂: C, 84.0;

Table 1. *Absorption spectra of compounds related to 7- and 12-methylbenz[a]anthracene*

Spectra were measured as described in the text. When measured, the molecular extinction coefficient is shown in parentheses (as log ϵ). *, Inflection.

Compound	$\lambda_{\max.}$ (m μ)
7-Methylbenz[a]anthracene	222, 233, 259, 271, 282, 292, 302, 320, 335, 351, 370 and 389
7-Acetoxyethylbenz[a]anthracene	222 (4.53), 233 (4.51), 259 (4.51), 271 (4.61), 281 (4.88), 292 (4.97), 302 (3.67), 321 (3.67), 337 (3.86), 352 (3.95), 370 (3.82) and 389 (3.01)
7-Hydroxymethylbenz[a]anthracene	222 (4.53), 233 (4.50), 259 (4.53), 271 (4.59), 280 (4.85), 291 (4.95), 302 (3.63), 320.5 (3.62), 335 (3.84), 351 (3.93), 368 (3.80) and 388 (3.02)
Benz[a]anthracene-7-carboxaldehyde	220, 232, 250, 292, 302, 382 and 398
Benz[a]anthracene-7-carboxylic acid	233, 260, 271, 280, 291, 302*, 321, 334, 350, 368 and 388
12-Acetoxy-7-methylbenz[a]anthracene	229 (4.31), 261 (4.58), 271 (4.61), 282 (4.85), 292 (4.90), 320 (3.60), 341.5 (3.85), 357 (3.95), 375 (3.82) and 394 (3.22)
12-Hydroxy-7-methylbenz[a]anthracene (or 7-methylbenzanthracenone)	235, 252, 282 and 338
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7-methylbenz[a]-anthracene	217 (4.46), 237 (4.34), 250 (4.49), 259.5 (4.65), 268 (4.69), 292* (4.09), 302 (4.15) and 311 (4.11)
Product, probably <i>trans</i> -5,6-dihydro-5,6-dihydroxy-7-methylbenz[a]anthracene	217, 237, 250*, 260, 268, 292*, 302 and 311
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7-hydroxymethylbenz[a]anthracene	218 (4.46), 237 (4.34), 250* (4.45), 259.5 (4.67), 267.5 (4.74), 290 (4.04), 302 (4.14) and 312 (4.11)
5,6-Epoxy-5,6-dihydro-7-methylbenz[a]-anthracene	222 (4.38), 254 (4.53), 263 (4.70), 273 (4.75), 295 (4.10), 307 (4.18), 320 (4.12), 332 (3.27) and 350 (3.21)
Product, probably <i>S</i> -(5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)glutathione	218, 262, 272, 292, 304, 316 and 346
Product, probably <i>S</i> -(7-methyl-5-benz[a]-anthracenyl)glutathione	218, 263, 272, 292, 304, 316 and 346
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene	224, 231, 260*, 268, 275*, 282*, 295, 308, 321.5, 349, 359 and 368
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-7-hydroxymethylbenz[a]anthracene	224, 231, 260*, 267, 275*, 280*, 294, 306, 320, 348, 359 and 368
12-Methylbenz[a]anthracene	222, 232, 260, 270, 280, 290, 337, 351, 365 and 391
12-Acetoxyethylbenz[a]anthracene	221 (4.52), 235 (4.34), 262 (4.55), 271 (4.66), 280 (4.88), 291 (4.90), 324* (3.68), 338 (3.83), 354 (3.86), 361* (3.76), 371 (3.68) and 393 (3.38)
12-Hydroxymethylbenz[a]anthracene	221 (4.51), 236 (4.32), 262 (4.54), 270 (4.63), 280 (4.87), 291 (4.93), 324* (3.65), 338 (3.81), 348 (3.87), 352 (3.87), 361 (3.75), 371* (3.69) and 392 (3.35)
7-Acetoxy-12-methylbenz[a]anthracene	232 (4.31), 262 (4.53), 272.5 (4.57), 282 (4.82), 292.5 (4.86), 325 (3.60), 343 (3.84), 358 (3.95), 376 (3.82) and 392 (3.18)
7-Hydroxy-12-methylbenz[a]anthracene (or 12-methylbenz[a]anthracenone)	225, 264, 270, 291, 300 and 350
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-12-methylbenz[a]-anthracene	220 (4.47), 248 (4.52), 257.5 (4.62), 265 (4.60), 285 (4.11), 296 (4.15) and 305 (4.11)
<i>trans</i> -5,6-Dihydro-5,6-dihydroxy-12-methylbenz[a]anthracene	220 (4.48), 248 (4.53), 257.5 (4.64), 264.5 (4.65), 285 (4.12), 296 (4.17) and 385 (4.11)
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-12-methylbenz[a]anthracene	223, 231, 266, 276*, 302, 313, 327, 350 and 368
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-12-hydroxymethylbenz[a]anthracene	223, 231, 266, 276*, 282*, 302, 313, 327, 350 and 369

H, 5.4%), and that from the slower-moving fraction 12-acetoxy-7-methylbenz[a]anthracene in needles, m.p. 152° (Found: C, 83.7; H, 5.2%). A mixture of the two products had m.p. 118–121°. Fieser & Hershberg (1938) obtained the 7-acetoxyethyl derivative from this oxidation and reported m.p. 153.5–154° (corr.) for the 12-acetoxy derivative synthesized by an alternative route.

When similarly oxidized, 12-methylbenz[a]anthracene yielded 7-acetoxy-12-methylbenz[a]anthracene in plates crystallized from ethanol, m.p. 195–196° (Found: C, 83.8;

H, 5.3%), and 12-acetoxyethylbenz[a]anthracene in pale yellow flat needles crystallized from ethanol, m.p. 137° (Found: C, 83.8; H, 5.1%). Newman (1938) reported m.p. 192.4–193.4° for the 7-acetoxy derivative. The 12-acetoxyethyl derivative was hydrolysed by brief boiling with 5% (w/v) KOH in methanol to yield 12-hydroxymethylbenz[a]anthracene, separating from aq. ethanol in pale green plates, m.p. 159–160° (Found: C, 88.2; H, 5.7; C₁₉H₁₄O requires C, 88.3; H, 5.5%).

When 7-acetoxy-12-methyl- and 12-acetoxy-7-methyl-

benz[*a*]anthracene were similarly hydrolysed and the products examined on thin-layer chromatograms, compounds were detected that were presumed to be 7-hydroxy-12-methyl- and 12-hydroxy-7-methyl-benz[*a*]anthracene (or the corresponding benz[*a*]anthracenones) together with a number of unidentified substances.

*Preparation of 5,6-substituted derivatives of 7- and 12-methylbenz[*a*]anthracene.* *cis*-5,6-Dihydro-5,6-dihydroxy-7-methylbenz[*a*]anthracene, prepared by the oxidation of the parent hydrocarbon with OsO₄ by the method of Cook & Schoental (1948), separated from benzene in needles, m.p. 195°. Newman & Blum (1964) reported m.p. 194–195°. *cis*-5,6-Dihydro-5,6-dihydroxy-12-methylbenz[*a*]anthracene was similarly prepared; it separated from benzene in needles, m.p. 166° (decomp.) (Found: C, 82.55; H, 5.9; C₁₉H₁₆O₂ requires C, 82.6; H, 5.8%). On the two-

dimensional thin-layer chromatograms described above, both compounds were decomposed with acid to yield phenols that are presumed to be the 5-hydroxy derivatives of the parent hydrocarbons.

The dihydrodihydroxy derivatives were each heated on a steam bath with Na₂Cr₂O₇ in acetic acid for 30 min. 7-Methylbenz[*a*]anthracene-5,6-quinone separated from aq. acetic acid in orange plates, m.p. 206–208° (decomp.) (Found: C, 83.5; H, 4.2; C₁₉H₁₂O₂ requires C, 83.8; H, 4.4%), and 12-methylbenz[*a*]anthracene-5,6-quinone separated from acetic acid in red needles with a metallic lustre, m.p. 210–212° (Found: C, 84.0; H, 4.5%).

The quinones were reduced with LiAlH₄ in ether by the method of Booth, Boyland & Turner (1950). 12-Methylbenz[*a*]anthracene-5,6-quinone yielded *trans*-5,6-dihydro-5,6-dihydroxy-12-methylbenz[*a*]anthracene, separating from

Table 2. *Properties on thin-layer chromatograms of compounds related to 7-methylbenz[*a*]anthracene*

Details are given in the text.

Compound	<i>R_F</i> in		Fluorescence in u.v. light	
	Benzene	Benzene-ethanol (19:1, v/v)	Immediately	After exposure to NH ₃
7-Methylbenz[<i>a</i>]anthracene*	0.98	0.98	Violet	Violet
7-Acetoxy-methylbenz[<i>a</i>]anthracene	0.62	0.87	Violet	Violet
7-Hydroxy-methylbenz[<i>a</i>]anthracene	0.22	0.41	Violet	Violet
Benz[<i>a</i>]anthracene-7-carboxaldehyde				
Benz[<i>a</i>]anthracene-7-carboxylic acid	0.00	0.01	Violet	Violet
12-Acetoxy-7-methylbenz[<i>a</i>]anthracene	0.64	0.86	Violet	Violet
12-Hydroxy-7-methylbenz[<i>a</i>]anthracene	0.62	0.83	Pale green	Yellow
Metabolite, probably 3-hydroxy-7-methylbenz[<i>a</i>]anthracene	0.31	—	Violet	Green
Metabolite, probably 4-hydroxy-7-methylbenz[<i>a</i>]anthracene	0.48	—	Violet	Pink
Product, probably 5-hydroxy-7-methylbenz[<i>a</i>]anthracene	0.49	0.92	Violet	Yellow
Product, probably 8-hydroxy-7-methylbenz[<i>a</i>]anthracene	0.42	—	Violet	Pink
Product, probably 9-hydroxy-7-methylbenz[<i>a</i>]anthracene	0.25	—	Violet	Green
Metabolite, possibly 1-hydroxy-7-hydroxymethylbenz[<i>a</i>]anthracene	0.00	0.46	Violet	Yellow
Metabolite, possibly 4-hydroxy-7-hydroxymethylbenz[<i>a</i>]anthracene	0.00	0.43	Blue violet	Orange
Metabolite, possibly 3-hydroxy-7-hydroxymethylbenz[<i>a</i>]anthracene	0.00	0.41	Blue violet	Green
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7-methylbenz[<i>a</i>]anthracene	0.00	0.39	Dark violet	Dark violet
Product, probably <i>trans</i> -5,6-dihydro-5,6-dihydroxy-7-methylbenz[<i>a</i>]anthracene	0.00	0.37	Dark violet	Dark violet
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-7-methylbenz[<i>a</i>]anthracene	0.00	0.25	Violet	Violet
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7-hydroxymethylbenz[<i>a</i>]anthracene	0.00	0.15	Dark violet	Dark violet
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-7-hydroxymethylbenz[<i>a</i>]anthracene	0.00	0.12	Violet	Violet

* *R_F* 0.45 in light petroleum (b.p. 80–100°)–benzene (19:1, v/v).

benzene in needles, m.p. 208° (Found: C, 82.9; H, 5.8; C₁₈H₁₆O₂ requires C, 82.6; H, 5.8%). 7-Methylbenz[a]anthracene 5,6-quinone yielded two products on thin-layer chromatograms, one of which was indistinguishable from the *cis*-dihydrodihydroxy compound. The second product, which had a similar absorption spectrum to the first, is presumed to be the *trans*-isomer (V). Both products yielded the expected 5-hydroxy-7-methylbenz[a]anthracene with acid on two-dimensional thin-layer chromatograms, and the *trans*-dihydrodihydroxy-12-methyl compound yielded the corresponding 5-hydroxy-12-methyl derivative under similar conditions. 7,12-Dimethylbenz[a]anthracene-5,6-quinone also yielded a mixture of *cis*- and *trans*-isomers with LiAlH₄ (Boyland & Sims, 1967).

5,6-Epoxy-5,6-dihydro-7-methylbenz[a]anthracene (II) was prepared by the method of Newman & Blum (1964). Under the conditions described by Boyland & Sims (1965b), the epoxide rearranged with HCl to yield a product with the chromatographic properties of 5-hydroxy-7-methylbenz[a]anthracene, reacted with water to yield a product with the properties of *trans*-5,6-dihydro-5,6-dihydroxy-7-methylbenz[a]anthracene (V) and reacted with *N*-acetylcysteine

or with GSH to yield products with the properties of the *N*-acetylcysteine derivative and GSH derivative (VI) respectively (see Table 4). These conjugates were rapidly decomposed by dilute mineral acids to yield mainly the parent hydrocarbon and the oxidized forms of the sulphur-containing amino acid side chains. Small amounts of products that are probably 7-methyl-5-benz[a]anthracenylmercapturic acid and the corresponding glutathione conjugate were also formed from the respective parent conjugates. The decomposition products were identified by means of thin-layer or paper chromatography.

7-Acetoxy-methylbenz[a]anthracene was oxidized with OsO₄ as before and the product was hydrolysed by brief boiling with KOH in methanol to yield *cis*-5,6-dihydro-5,6-dihydroxy-7-hydroxymethylbenz[a]anthracene separating in needles from aq. ethanol, m.p. 228° (decomp.) (Found: C, 77.9; H, 5.5; C₁₉H₁₆O₃ requires C, 78.1; H, 5.5%).

Experiments with rat-liver homogenates

These were carried out as described by Sims (1966); in each experiment 10mg. of substrate was incubated with

Table 3. *Properties on thin-layer chromatograms of compounds related to 12-methylbenz[a]anthracene*

Details are given in the text.

Compound	<i>R_F</i> in		Fluorescence in u.v. light	
	Benzene	Benzene-ethanol (19:1, v/v)	Immediately	After exposure to NH ₃
12-Methylbenz[a]anthracene*	0.98	0.98	Violet	Violet
12-Acetoxy-methylbenz[a]anthracene	0.62	0.87	Violet	Violet
12-Hydroxymethylbenz[a]anthracene	0.40	0.65	Violet	Violet
7-Acetoxy-12-methylbenz[a]anthracene	0.62	0.86	Violet	Violet
7-Hydroxy-12-methylbenz[a]anthracene	0.60	8.84	Green	Green
Metabolite, probably 3-hydroxy-12-methylbenz[a]anthracene	0.31	—	Violet	Green
Metabolite, probably 4-hydroxy-12-methylbenz[a]anthracene	0.47	—	Violet	Pink
Product, probably 5-hydroxy-12-methylbenz[a]anthracene	0.48	0.90	Violet	Yellow
Product, probably 8-hydroxy-12-methylbenz[a]anthracene	0.43	—	Violet	Pink
Product, probably 9-hydroxy-12-methylbenz[a]anthracene	0.28	—	Violet	Green
Metabolite, possibly 1-hydroxy-12-hydroxymethylbenz[a]anthracene	0.00	0.47	Violet	Yellow
Metabolite, possibly 4-hydroxy-12-hydroxymethylbenz[a]anthracene	0.00	0.45	Violet	Orange
Metabolite, possibly 3-hydroxy-12-hydroxymethylbenz[a]anthracene	0.00	0.42	Violet	Green
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-12-methylbenz[a]anthracene	0.00	0.39	Dark violet	Dark violet
<i>trans</i> -5,6-Dihydro-5,6-dihydroxy-12-methylbenz[a]anthracene	0.00	0.37	Dark violet	Dark violet
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-12-methylbenz[a]anthracene	0.00	0.26	Violet	Violet
Metabolite, probably 8,9-dihydro-8,9-dihydroxy-12-hydroxymethylbenz[a]anthracene	0.00	0.13	Violet	Violet

* *R_F* 0.45 in light petroleum (b.p. 80–100°)–benzene (19:1, v/v).

Table 4. *Properties on paper chromatograms of amino acid conjugates derived from 7-methylbenz[a]anthracene*

Details are given in the text. ++, Strong, and +, weak, yellow colour on pink background.

Probable identity of conjugate	R_F in solvent		Fluorescence in in u.v. light	Colour with ninhydrin	Reaction with the platonic iodide reagent*
	(1)	(2)			
<i>N</i> -Acetyl- <i>S</i> -(5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)cysteine	0.36	0.84	Dark violet	None	++
7-Methyl-5-benz[a]anthracenylmercapturic acid	0.53	0.87	Violet	None	+
<i>S</i> -(5,6-Dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)glutathione	0.12	0.47	Dark violet	Purple	++
<i>S</i> -(7-Methyl-5-benz[a]anthracenyl)glutathione	0.21	0.62	Violet	Purple	+
<i>S</i> -(5,6-Dihydro-6-hydroxy-12-methyl-5-benz[a]anthracenyl)glutathione	0.11	0.47	Violet	Purple	++

* Method of Toennies & Kolb (1951).

homogenate prepared from the livers of four rats. The ethyl acetate-soluble products were separated on thin-layer chromatograms and identified by their chromatographic properties and absorption spectra as indicated below by using the methods described by Sims (1966). GSH conjugates were separated from the reaction mixtures by means of activated charcoal as described by Boyland & Sims (1965b), and examined on paper chromatograms. The properties on thin-layer chromatograms of products related to 7-methylbenz[a]anthracene (I) are recorded in Table 2 and the properties of those related to 12-methylbenz[a]anthracene in Table 3. The properties of products examined on paper chromatograms are recorded in Table 4.

RESULTS

The results are outlined in Scheme 1; roman numerals refer to this Scheme.

Metabolism of 7-methylbenz[a]anthracene (I). 7-Hydroxymethylbenz[a]anthracene (IV) was identified in the ethyl acetate-soluble products of the metabolism of the hydrocarbon by its properties on thin-layer chromatograms and by its absorption spectrum. Two phenolic products were also present in small amounts; they were not positively identified, but were presumed to be 3- and 4-hydroxy-7-methylbenz[a]anthracene because of the similarity of their chromatographic properties with those of 3- and 4-hydroxybenz[a]anthracene (Boyland & Sims, 1964b). Three other slower-moving phenolic products were also detected; they were also formed in the metabolism of the 7-hydroxymethyl derivative (IV) described below.

Three dihydrodihydroxy compounds were also detected. The product moving the fastest on thin-layer chromatograms was indistinguishable in its chromatographic properties and in its absorption spectrum from the synthetic compound that is probably *trans*-5,6-dihydro-5,6-dihydroxy-7-methylbenz[a]anthracene (V). On acid-treated two-dimensional thin-layer chromatograms it

yielded a phenol indistinguishable from 5-hydroxy-7-methylbenz[a]anthracene.

The second compound was tentatively identified as 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene (III) (or possibly the 10,11-dihydro-10,11-dihydroxy isomer) because its chromatographic properties and absorption spectrum were similar to those of 8,9-dihydro-8,9-dihydroxybenz[a]anthracene (Boyland & Sims, 1964b). On acid-treated two-dimensional thin-layer chromatograms it yielded two phenols that were tentatively identified as 8- and 9-hydroxy-7-methylbenz[a]anthracene. That believed to be the 9-hydroxy isomer formed the largest spot on the chromatograms.

The third compound, present in small amounts, had an absorption spectrum similar to that of the second. Since it also appeared to be a metabolite of 7-hydroxymethylbenz[a]anthracene, it was tentatively identified as 8,9-dihydro-8,9-dihydroxy-7-hydroxymethylbenz[a]anthracene (VII).

An examination of the aqueous phase from one of these oxidations showed the presence of a ninhydrin-positive product with the mobility on paper chromatograms of *S*-5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenylglutathione (VI). The product was purified on a paper chromatogram developed with solvent (1), and the material eluted with methanol from the appropriate strip cut from the chromatogram was acidified with hydrochloric acid. Products with the chromatographic properties of 7-methylbenz[a]anthracene, *S*-(7-methyl-5-benz[a]anthracenyl)glutathione and GSSG were detected.

Metabolism of 7-hydroxymethylbenz[a]anthracene (IV). Five metabolic products of the hydroxymethyl derivatives were detected in the ethyl acetate fraction. Three of these were chromatographically indistinguishable from the slower-moving phenolic metabolites of the hydrocarbon.

Scheme 1. Probable metabolic pathways of 7-methylbenz[a]anthracene. Those of 12-methylbenz[a]anthracene are similar.

They were not identified, but it is possible that they are 1-, 3- and 4-hydroxy-7-hydroxymethylbenz[a]anthracene.

The major metabolite was the dihydrodihydroxy compound previously detected as a metabolite of the parent hydrocarbon. The absorption spectrum closely resembled that of the product tentatively identified as 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene, so that the metabolite is probably the 7-hydroxymethyl derivative (VII). After treatment with acid on two-dimensional chromatograms it yielded a phenol that is probably 9-hydroxy-7-hydroxymethylbenz[a]anthracene.

The fifth metabolite, which was present in very small amounts, possessed the chromatographic properties and absorption spectrum of benz[a]-

anthracene-7-carboxylic acid. Neither the corresponding aldehyde nor 5,6-dihydro-5,6-dihydroxy-7-hydroxymethylbenz[a]anthracene was detected as a metabolite of the 7-hydroxymethyl derivative.

Metabolism of 5,6-epoxy-5,6-dihydro-7-methylbenz[a]anthracene (II). The ethyl acetate-soluble fraction was shown to contain products with the chromatographic properties of *trans*-5,6-dihydro-5,6-dihydroxy-7-methylbenz[a]anthracene and of 5-hydroxy-7-methylbenz[a]anthracene. The aqueous phase was shown by paper chromatography to contain a product with the chromatographic properties and absorption spectrum of *S*-(5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)glutathione (VI). With hydrochloric acid, the metabolite was decomposed into products with the

properties of 7-methylbenz[*a*]anthracene, *S*-(7-methyl - 5 - benz[*a*]anthracenyl)glutathione and GSSG.

Metabolism of benz[*a*]anthracene-7-carboxaldehyde. The aldehyde was converted by rat-liver homogenates into products with the properties of the 7-hydroxymethyl derivative (IV) and the phenols and the 8,9-dihydro-8,9-dihydroxy derivative (VII) related to the hydroxymethyl derivative. A product with the properties of benz[*a*]anthracene-7-carboxylic acid was also detected; a much higher proportion of the acid was formed in these experiments than in those with the 7-hydroxymethyl derivative.

Metabolism of 7-acetoxymethylbenz[*a*]anthracene. The 7-acetoxy derivative was readily deacetylated by rat-liver homogenates to yield a compound with the properties of the parent hydroxymethyl derivative (IV), together with all the metabolites of the hydroxymethyl derivative described above.

Metabolism of 12-methylbenz[*a*]anthracene. The metabolic oxidation of 12-methylbenz[*a*]anthracene yielded the same types of products as that of the 7-methyl isomer. 12-Hydroxymethylbenz[*a*]anthracene was identified by its absorption spectrum and chromatographic properties. Two phenols closely resembling the corresponding 7-methylbenz[*a*]anthracene metabolites in their chromatographic properties were detected; they were presumed to be 3- and 4-hydroxy-12-methylbenz[*a*]anthracene.

Three slower-moving phenolic products appeared to be related both to 12-hydroxymethylbenz[*a*]anthracene, since they are also metabolites of this compound, and to the phenolic products derived from 7-hydroxymethylbenz[*a*]anthracene.

Of the three dihydrodihydroxy compounds detected, one was indistinguishable from *trans*-5,6-dihydro-5,6-dihydroxy-12-methylbenz[*a*]anthracene in its chromatographic properties and absorption spectrum, the second was tentatively identified as 8,9-dihydro-8,9-dihydroxy-12-methylbenz[*a*]anthracene because of its close resemblance in chromatographic properties and absorption spectrum to the corresponding 7-methyl derivative, and the third appeared to be 8,9-dihydro-8,9-dihydroxy-12-hydroxymethylbenz[*a*]anthracene.

Occasionally, small amounts of a product with the chromatographic properties of 7-hydroxy-12-methylbenz[*a*]anthracene were also detected.

An examination of the aqueous layer showed the presence of a ninhydrin-positive product that had properties similar to those of the GSH conjugate derived from 7-methylbenz[*a*]anthracene; it is probably *S*-(5,6-dihydro-6-hydroxy-12-methyl-5-benz[*a*]anthracenyl)glutathione.

Metabolism of 12-hydroxymethylbenz[*a*]anthracene. The metabolism of this compound by rat-liver

homogenates was similar to that of the 7-hydroxymethyl isomer. Three phenolic derivatives, possibly 1-, 3- and 4-hydroxy-12-methylbenz[*a*]anthracene, and a dihydrodihydroxy compound with the chromatographic properties and the absorption spectrum of the 12-methylbenz[*a*]anthracene metabolite that is believed to be 8,9-dihydro-8,9-dihydroxy-12-methylbenz[*a*]anthracene, were detected. A small amount of a product that had properties similar to benz[*a*]anthracene-7-carboxylic acid, and was presumed to be the 12-carboxylic acid, was also detected.

DISCUSSION

Both 7- and 12-methylbenz[*a*]anthracene induce sarcomas when tested by subcutaneous injection into mice (Clayson, 1962), but neither compound appears to be as active as 7,12-dimethylbenz[*a*]anthracene. The dimethyl compound differs from the monomethyl compounds in its ability to induce adrenal necrosis in rats (Huggins & Morii, 1961). 7-Hydroxymethylbenz[*a*]anthracene induces local sarcomas after subcutaneous injection into mice (Badger *et al.* 1940), but is not effective in inducing adrenal necrosis in rats (Boyland, Sims & Huggins, 1965); both 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 12-hydroxymethyl-7-methylbenz[*a*]anthracene induce local sarcomas in mice, but only the 7-hydroxymethyl-12-methyl derivative induces adrenal necrosis in rats (Boyland *et al.* 1965). 12-Hydroxymethylbenz[*a*]anthracene has not been tested for biological activity.

In spite of the differences in biological activities between the monomethylbenzanthracenes and their hydroxymethyl derivatives and the dimethyl compound and its monohydroxymethyl derivatives, the metabolic pathways of all the compounds are similar, except that products formed by reaction on the 5,6-bonds (the 'K regions') of the monomethylbenzanthracenes have been identified, whereas, in spite of a careful search, none has been found with 7,12-dimethylbenz[*a*]anthracene (Boyland & Sims, 1965*a*, 1967).

The lead tetra-acetate and osmium tetroxide oxidations show that, like 7,12-dimethylbenz[*a*]anthracene, both the 7- and 12-positions (the 'L regions') and the 5,6-bond (the 'K region') of the monomethyl derivatives are active chemically. The ozonolysis of these derivatives has not been examined, but Moriconi & Taranko (1963) have shown that in the ozonolysis of the dimethyl derivative there is a decrease in activity at the 'L region' and an increase at the 'K region', relative to benz[*a*]anthracene; presumably the activities of the monomethyl derivatives would be intermediate. It is therefore difficult to see why there is an apparent absence of metabolic reactions involving the 'K

region' of the dimethyl derivative; possibly the 'K region' is involved in binding to cell constituents such as proteins and nucleic acids. 'K-region' products have been found with benz[a]anthracene and dibenz[a,h]anthracene (Boyland & Sims, 1964b, 1965b), pyrene (Boyland & Sims, 1964a) and 3-methylcholanthrene (Sims, 1966), but not with benzo[a]pyrene (Sims, 1967). The formation of products on the 'K region' presumably involves epoxide intermediates; 5,6-epoxy-5,6-dihydro-7-methylbenz[a]anthracene yields essentially the same products as those formed on the 'K region' in the metabolism of the parent hydrocarbon.

Like 7,12-dimethylbenz[a]anthracene and its monohydroxymethyl derivatives, the monomethylbenz[a]anthracenes and their hydroxymethyl derivatives all yield phenols, apparently arising by hydroxylation mainly at the 3- and 4-positions, and dihydrodihydroxy compounds, apparently formed on the 8,9-bond (or, less likely, the 10,11-bond). Very little oxidation of the methyl or hydroxymethyl groups to carboxylic acids occurred under the conditions used in this work, and the intermediate aldehydes could not be detected.

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