The Metabolism of 1,2-Benzanthracene in Mice and Rats*

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With an appendix on absorption spectra by E. R. Holiday, M. A., B. M.**

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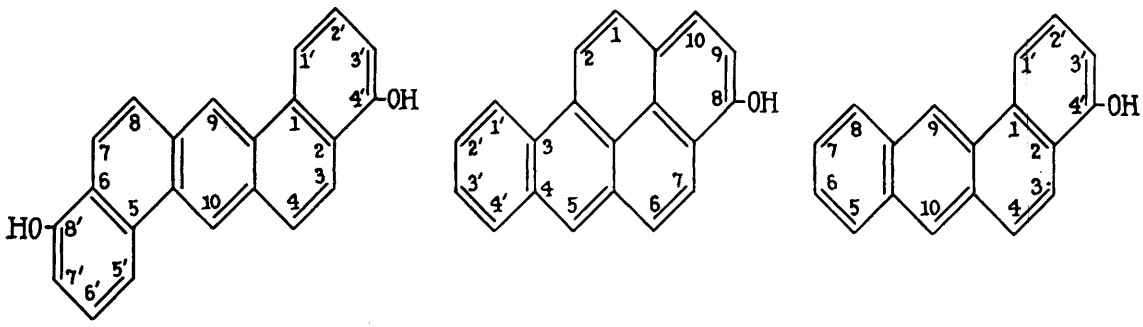
From previous metabolic studies it is known that carcinogenic hydrocarbons can be converted by the animal body into phenolic derivatives. In mice and rats, 1,2,5,6-dibenzanthracene is converted into a dihydroxy derivative (3, 8), possessing properties similar to those of synthetic 4',8'-dihydroxy-1,2,5,6-dibenzanthracene, formula I (4), while 3,4-benzpyrene is converted into a monohydroxy derivative (11, 5, 6, 7), which, from recent studies (2), appears to be the 8-hydroxy compound, formula II.

A similarity in the positions of the hydroxy groups in the two cases becomes apparent when the respective parent hydrocarbons are considered as derivatives of 1,2-benzanthracene; compare formulas I, II, and III. From this it might be expected that 1,2-benzanthracene itself, when injected into mice or rats, should be converted into its 4'-hydroxy derivative, formula III. The purpose of the present investigation was to establish whether this is actually the case.



I II III

EXPERIMENTAL

The experimental procedure for the isolation of the metabolite was similar to that used previously by the authors in the study of the metabolism of 3,4-benzpyrene (1). About 2 ml. of a saturated solution of 1,2-benzanthracene in arachis oil was injected intraperitoneally per rat, and 0.4 ml. of the solution per mouse.

For 10 to 14 days after injection, the feces were collected, dried, and ground to a fine powder. This powder was extracted with cold benzene by percolation, and the pooled benzene extracts were passed through columns of alumina.[1] The columns were developed with excess of benzene, and in each case the zone showing a strong bluish white fluorescence in ultraviolet light was cut and eluted with methanol. After evaporation, the residue was methylated with dimethyl sulfate in the presence of aqueous NaOH, and the product transferred into benzene. The benzene solution was dried with anhydrous Na_2SO_4, and then passed through another column of alumina. The fluorescent filtrate, containing the methylated metabolite, was evaporated to dryness, and sublimed in high vacuum. The sublimate consisted of an oily pale yellow material, showing a tendency to crystallize.

The phenolic nature of the metabolite (*i.e.*, before methylation) was indicated by its chromatographic behavior from different solvents, and by its solubility in strong alkali, the latter being accompanied by the

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[1] "Aluminum oxide for adsorption purposes," British Drug Houses, Ltd., London.

characteristic change in fluorescence from bluish violet to yellow.

On oxidation with chromic acid it yielded a yellow product. This was not isolated, but it was found to differ from the 9,10-quinone (obtained by oxidation of benzanthracene itself with chromic acid) by its stronger adsorbability when a mixture of the two was passed through alumina. This result is compatible with the view that the oxidized yellow product of the metabolite is a hydroxyquinone and, therefore, that the OH group in the metabolite was not in the 9- or 10- position.

As in the study of the metabolism of 3,4-benzpyrene (1), conversion of the phenolic metabolite into its more stable methylated derivative proved advantageous as a practical expedient for its identification. For comparison, the following methoxy derivatives of 1,2-benzanthracene were synthesized.

4'-Methoxy-1,2-benzanthracene.—Prepared by methylation of the 4'-hydroxy compound, produced according to the method of Sempronj (12). The product was purified by chromatography and crystallization, yielding colorless needles; m.p. 160–161° C.

Analysis (Strauss and Weiler).—10.72 per cent OCH_3 (Theor. 12.02 per cent).

9,10-Dimethoxy-1,2-benzanthracene.—Prepared by reductive methylation of the 9,10-quinone, followed by purification by chromatography and crystallization. The final product consisted of colorless rhombic crystals; m.p. 137–138° C.

Analysis (Strauss and Weiler).—20.7 per cent OCH_3 (Theor. 21.5 per cent).

The 3-methoxy-1,2-benzanthracene, synthesized by Fieser and Dietz (9), was not prepared for direct comparison, as the description of its absorption spectrum by Jones (10) made it possible to establish whether or not it was identical with the methylated metabolite under investigation.

Chromatographic behavior from mixtures, fluorescence spectra, and ultraviolet absorption spectra were used as criteria for identification.

Chromatographic behavior.—Tests for identity or nonidentity of two substances can often be performed by passing the mixture of the two through chromatographic columns, and developing the columns with a suitable solvent. The resolution of the mixture into two separate zones is proof of nonidentity. When separation into two distinct zones does not occur, proof of nonidentity can still be obtained sometimes by testing successive samples of eluate from a fluid chromatogram for characteristic properties. While chromatographic resolution of a mixture is proof of nonidentity of its constituents, failure of resolution, though indicative, is not proof of identity.

Such tests were carried out with mixtures of the methylated metabolite and the synthetic compounds mentioned above. While mixtures of the methylated metabolite with the 9,10-dimethoxy compound could be resolved into the two components, no such resolution was found possible from mixtures of the methylated metabolite and synthetic 4'-methoxy-1,2-benzanthracene.

Fluorescence spectra.—This method proved to be very helpful in obtaining evidence of nonidentity between related derivatives of polycyclic hydrocarbons, and in indicating identity where the spectra were the same. In view of the relative simplicity of the technic, its high sensitivity, and its relative specificity in the presence of impurities (especially if the latter were themselves nonfluorescent), this method, together with chromatographic analysis, was used with great advantage during the preliminary stages of extraction, synthesis, or purification.

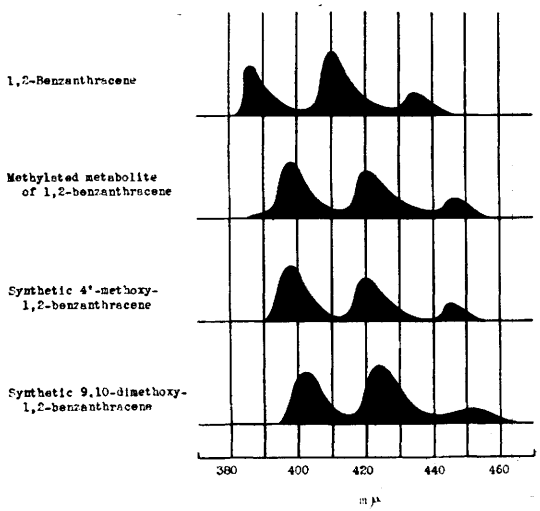

FIG. 1.—Semiquantitative representation of fluorescence spectra of 1,2-benzanthracene and some of its metabolic (methylated) and synthetic derivatives, in benzene.

Semiquantitative representations of the fluorescence spectra of benzanthracene, its 4'- and 9,10-methoxy derivatives, and the methylated metabolite, are shown in Fig. 1. It will be seen that the methylated metabolite, while differing in its fluorescence spectrum from the parent hydrocarbon and from the 9,10-dimethoxy compound, is indistinguishable from the 4'-methoxy compound.

Ultraviolet absorption spectra.—With the class of compounds under investigation (*i.e.*, those possessing multiple absorption band systems with fine structure), this method constitutes a reliable means of identification.

Comparisons were made by Dr. Holiday between the absorption spectra of the methylated metabolite and those of the other methoxy derivatives of benz-

anthracene available or described in the literature. The close agreement between the absorption spectra of the methylated metabolite and of the synthetic 4′-methoxy-1,2-benzanthracene (see appendix) suggested that they were identical.

From these results it may be inferred that the metabolite excreted in the feces was 4′-hydroxy-1,2-benzanthracene.

DISCUSSION

The results so far obtained in the studies on the metabolism of carcinogens in mice and rats appear to conform to a certain pattern. While considerably more evidence is required before the chemical mechanism involved can be understood, some tentative conclusions seem justifiable at this stage.

1. There appears to be a similarity in the positions of metabolic oxidation in the molecule as far as 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 1,2-benzanthracene are concerned; compare formulas I, II, and III.

2. The positions in the molecule metabolically attacked are not those that are most reactive chemically (the latter being the 9,10- positions in the case of benzanthracene and dibenzanthracene, and the 5- position in the case of benzpyrene). This point has already been stressed by Cason and Fieser (4).

3. It is interesting to observe that with benzanthracene and dibenzanthracene, the positions in the molecule metabolically attacked (4′- and 4′,8′- respectively) are also those where sulfonation occurs *in vitro, provided the most reactive positions (9,10-) are blocked, as in the case of quinones.*

4. These results are compatible with the view that in the process of metabolic oxidation some group, possibly an enzyme, blocks the most reactive position in the molecule, so that metabolic oxidation occurs in the *next* reactive positions.

SUMMARY

A fluorescent phenolic derivative has been isolated from the feces of mice and rats injected with 1,2-benzanthracene by the intraperitoneal route.

On methylation of the metabolite a product was obtained that possessed identical chromatographic behavior, fluorescence spectrum, and absorption spectrum (in the range longer than 300 mμ) with those of synthetic 4′-methoxy-1,2-benzanthracene.

It is suggested, therefore, that the metabolite is 4′-hydroxy-1,2-benzanthracene.

The mechanism of metabolic oxidation of carcinogenic hydrocarbons is discussed briefly.

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REFERENCES

1. Berenblum, I., and Schoental, R. The Metabolism of 3,4-Benzpyrene in Mice and Rats. I. The Isolation of a Hydroxy and a Quinone Derivative, and a Consideration of Their Biological Significance. Cancer Research, **3**: 145-150. 1943.
2. Berenblum, I., Crowfoot, D., Holiday, E. R., and Schoental, R. The Metabolism of 3,4-Benzpyrene in Mice and Rats. II. The Identification of the Isolated Products as 8-Hydroxy-3,4-Benzpyrene and 3,4-Benzpyrene-5,8-Quinone. Cancer Research, **3**:151-158. 1943.
3. Boyland, E., Levi, A. A., Mawson, E. H., and Roe, E. Metabolism of Polycyclic Compounds. 4. Production of a Dihydroxy-1:2:5:6-Dibenzanthracene from 1:2:5:6-Dibenzanthracene. Biochem. J., **35**:184-191. 1941.
4. Cason, J., and Fieser, L. F. Synthesis of 4′,8′-Dihydroxy-1,2,5,6-Dibenzanthracene and Its Relation to Products of Metabolism of the Hydrocarbon. J. Am. Chem. Soc., **62**:2681-2687. 1940.
5. Chalmers, J. G. The Elimination of 3:4-Benzpyrene and Other Polycyclic Hydrocarbons from the Mouse. Biochem. J., **32**:271-278. 1938.
6. Chalmers, J. G. Elimination of 3:4-Benzpyrene from the Rat. Biochem. J., **34**:678-684. 1940.
7. Chalmers, J. G., and Crowfoot, D. The Elimination of 3:4-Benzpyrene from the Animal Body after Subcutaneous Injection. 2. Changed Benzpyrene. Biochem. J., **35**:1270-1275. 1941.
8. Dobriner, K., Rhoads, C. P., and Lavin, G. I. The Spectroscopic Study of Biological Extracts. II. The Detection, Isolation, and Biological Effects of the Metabolites of 1,2,5,6-Dibenzanthracene. Cancer Research, **2**:95-107. 1942.
9. Fieser, L. F., and Dietz, E. M. 1,2-Benz-3,4-Anthraquinone. J. Am. Chem. Soc., **51**:3141-3148. 1929.
10. Jones, R. N. Further Observations on the Absorption Spectra of Derivatives of 1,2-Benzanthracene. J. Am. Chem. Soc., **63**:151-155. 1941.
11. Peacock, P. R. Evidence Regarding the Mechanism of Elimination of 1:2-Benzpyrene, 1:2:5:6-Dibenzanthracene, and Anthracene from the Blood-Stream of Injected Animals. Brit. J. Exper. Path., **17**:164-172. 1936.
12. Sempronj, A. Alcuni derivati dell'acido 1,2-benzantrachinon-4′-solfonico. Gazz. chim. ital., **69**:448-453. 1939.