*SELECTIVE DESTRUCTION OF ADRENAL CORTEX BY PULSE DOSES OF DERIVATIVES OF 12-METHYLBENZ(a)ANTHRACENE**

By Charles Huggins, Sotokichi Morii,† and John Pataki

BEN MAY LABORATORY FOR CANCER RESEARCH, UNIVERSITY OF CHICAGO

Communicated January 21, 1969

Abstract.—Pulse doses of emulsions of ten polynuclear aromatic hydrocarbons were found to destroy the adrenal cortex selectively.

The corticolytic hydrocarbons are derivatives of 12-methylbenz(a)anthracene which possess at position 7 an alkyl, methoxymethyl, formyl, or hydroxyalkyl group. It would appear that the active corticolytic agent is 7-hydroxyalkyl-12-methylbenz(a)anthracene, of chemical source or generated in metabolism.

This paper deals with the preparation of lipid emulsions of polynuclear aromatic hydrocarbons and the effect on the adrenal cortex when such an emulsion is injected intravenously as a pulse dose.[1] It was found that the corticolytic hydrocarbons are derivatives of 12-methylbenz(a)anthracene.‡

The middle layer of the adrenal gland, comprising zona fasciculata and zona reticularis, acts as a functional unit in synthesizing C_{21} steroids, cortisol, and corticosterone. It can be destroyed by members of two widely different chemical categories, and the destructive effects can be prevented by suitable treatments appropriate to each class of compound. (1) 7,12-DMBA[2] and a few of its congeners,[3] when fed to adult rats, selectively destroy the adrenal cortex after about 24 hours. The lethal effect of 7,12-DMBA can be forestalled by prior administration to the animal of any of a large number of aromatics.[4–6] (2) Long electropositive polymers, such as hexadimethrine,[7] containing quaternary ammonium ions, cause necrosis and hemorrhage in adrenal, liver, and other organs within a few minutes. This unspecific effect can be prevented by administering long electronegative compounds[8] to the animal, whereas pretreatment with aromatics has no protective influence.

7,12-DMBA is distinctive[9] in its metabolism. Many polycyclic aromatic hydrocarbons are metabolized by oxidation of the more reactive double bonds of the ring system; in contrast, the methyl groups of this derivative are the most reactive molecular sites. The main products[9] of the oxidation of 7,12-DMBA by liver homogenates or lead tetraacetate or the Fe^{++}-ascorbic acid-O_2 hydroxylating system are the isomeric monohydroxyl derivatives. In an earlier experiment,[10] solutions of this derivative and of its principal metabolites were fed to rats to investigate possible destruction of the adrenal. We found that 7-hydroxymethyl-12-Me-BA was more active than 7,12-DMBA as a corticolytic agent, whereas 12-hydroxymethyl-7-Me-BA did not attack the adrenal cortex.

Materials and Methods.—Chemical: Of the 25 compounds tested, 9 were new and were synthesized in our laboratories. The new compounds are: VI, XIII, XIV, XVII, XVIII, XIX, XXII, XXIII, XXIV (Tables 1 and 2). Satisfactory elemental analyses were obtained for each new compound. Their nuclear magnetic resonance spectra are

also in complete agreement with the assigned structures. The compounds migrated as a single spot in thin-layer chromatography in a mixture of benzene and cyclohexane (1:4).

Preparations of emulsions: Emulsions containing hydrocarbons, 0.1–1% w/v, were prepared. The solubility of each compound in cottonseed oil was determined, since this value set the upper limit of the amount of hydrocarbon in the emulsion. The final concentration of hydrocarbon was about 10% of its solubility in oil. For example, if the solubility of hydrocarbon in cottonseed oil at room temperature was 1%, an emulsion of hydrocarbon, 0.1% w/v, could be prepared.

First, cottonseed oil was emulsified in water containing lecithin and a nonionic detergent; it was prepared by the method of Schurr[11] to form a 15% w/w emulsion. The hydrocarbon was then incorporated in the Schurr emulsion.

Over gentle heat, the hydrocarbon was dissolved in DMSO in a flask jacketed in aluminum and containing a magnetic spin bar. The Schurr emulsion was added dropwise until the final concentration of DMSO was 5%. A reflux condenser was attached to the flask, and the mixture was boiled for 1 hr with constant *slow* stirring.

After the emulsion had cooled, a drop was placed on a glass slide, covered with a slip, and examined with a microscope equipped with a calibrated ocular micrometer. If no crystals were present, the maximum diameter of the fat droplets was measured. Drops were also placed in both chambers of a hemocytometer and fat globules larger than 5 μ in diameter were counted within the lined area (9 mm^2). In the 33 emulsions that we prepared, the total number of fat droplets per chamber was 29 $\pm$ 18 droplets larger than 5 μ and 9 $\pm$ 6 greater than 15 μ in diameter. These emulsions were injected without causing respiratory distress or other symptoms in the recipient.

Results.—Adrenal hemorrhage evoked by aromatic hydrocarbons is impressive on gross examination on day 3. As extravasated blood is absorbed, massive necrosis in the middle layer of the adrenal becomes apparent (Fig. 1).

Derivatives of 7-methylbenz(a)anthracene: Seven compounds (Table 1) in this class were examined for their ability to incite adrenal hemorrhage; only one of them, 7,12-DMBA (IV), was active in this regard. The nature of the substituent (R) at position 12 (Fig. 2) of derivatives of 7-Me-BA is critical for corticolytic activity; a methyl side chain at C_{12} is mandatory. The adrenal was not injured when R = H (I); hydroxymethyl (V); formyl (VI); ethyl (VII).

Two dimethyl derivatives, 6,7-DMBA (II) and 7,11-DMBA (III), were inactive, whereas 7,12-DMBA (IV) had high activity in causing selective adrenal destruction.

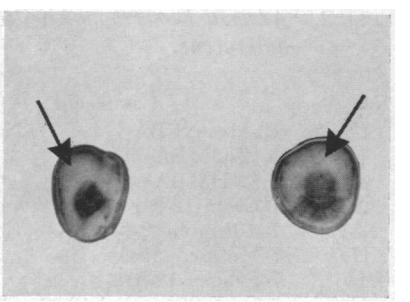

FIG. 1.—Equatorial sections of adrenal glands of a rat on day 11 following a pulse dose of 2 mg of 7-hydroxymethyl-12-Me-BA. Arrows denote areas of necrosis.

Derivatives of 12-methylbenz(a)anthracene: Nine derivatives of *12*-Me-BA (Table 2), in addition to 7,12-DMBA (IV), were corticolytic. Two side chains are required selectively to destroy the adrenal cortex; 12-methyl-BA (VIII) did not damage the adrenal. Derivatives of 12-Me-BA (Fig. 3) are corticolytic when R_1 = methyl (IV); hydroxymethyl (XV); formyl (XVI); ethyl (XI); 1-hydroxyethyl (XVII); 2-hydroxyethyl (XX); 1-hydroxy-*n*-propyl (XVIII). Three dimethyl derivatives—5,12-DMBA (IX), 6,12-DMBA (X), and 8,12-DMBA (XXI)—were inactive.

TABLE 1. *Adrenal hemorrhage elicited by pulse doses of derivatives of 7-methylbenz(a)-anthracene.*

No.	Compound	Melting point	Dose (mg)	Adrenal hemorrhage
I	7-Methyl-BA	139–140	7.5	0/4
II	6,7-DMBA	112–114	15	0/8
III	7,11-DMBA	143.5–145	15	0/4
IV	7,12-DMBA	122–123	4	16/16
V	7-Me-12-hydroxymethyl-BA	162–163.5	7.5	0/6
VI	7-Me-12-formyl-BA	129–131	15	0/14
VII	7-Me-12-ethyl-BA	76–78	25	0/8

It is noteworthy that 7-methoxymethyl-12-Me-BA (XIX) was highly active, whereas 7-methoxy-12-Me-BA (XIII) and 7-ethoxy-12-Me-BA (XIV) were inactive. Moreover 6,7,12-TMBA (XXIII) and 7,8,12-TMBA (XXV) were active compounds, whereas 5,7,12-TMBA (XXII) and 6,8,12-TMBA (XXIV) were inactive.

Discussion.—Injection of a pulse dose of a concentrated emulsion of a polynuclear aromatic hydrocarbon is a simple and useful method of introducing a considerable amount of a lipid rapidly into the blood. Many of the aromatic hydrocarbons have strong biological activity and incite impressive effects, *inter alia* massive but selective adrenal destruction and instant cancer. An additional advantage is the economy of compounds in scarce supply.

All the corticolytic hydrocarbons are derivatives of benz(a)anthracene and possess two active side chains situated at C_7 and C_{12}. A requirement of the corticolytic aromatics is an intact methyl group at position 12. But at position 7 in the 12-methylbenz(a)anthracene molecule, eight sorts of substituents are possible.

The most powerful corticolytic aromatic was 7-hydroxymethyl-12-Me-BA, followed in rank by 7,12-DMBA. It is known that hydroxymethyl groups are

TABLE 2. *Adrenal hemorrhage elicited by pulse doses of derivatives of 12-methylbenz(a)-anthracene.*

No.	Compound	Melting point	Dose (mg)	Adrenal hemorrhage
VIII	12-Methyl-BA	139–139.5	7.5	0/4
IX	5,12-DMBA	91.5–93	15	0/8
X	6,12-DMBA	75–76	15	0/8
XI	7-Ethyl-12-Me-BA	69.5–70.5	15	10/16
XII	7-*n*-Propyl-12-Me-BA	102–103.5	15	0/8
XIII	7-Methoxy-12-Me-BA	76–77.5	15	0/6
XIV	7-Ethoxy-12-Me-BA	118.5–119.5	15	0/6
XV	7-Hydroxymethyl-12-Me-BA	164–166	2	14/14
XVI	7-Formyl-12-Me-BA	113–114	5	6/8
XVII	7-(1-Hydroxyethyl)-12-Me-BA	124–126	5	6/6
XVIII	7-(1-Hydroxy-*n*-propyl)-12-Me-BA	69–69.5	15	2/6
XIX	7-Methoxymethyl-12-Me-BA	119–121	5	14/14
XX	7-(2-Hydroxyethyl)-12-Me-BA	125–128	5	4/6
XXI	8,12-DMBA	135–137	15	0/8
XXII	5,7,12-TMBA	129–131	15	0/10
XXIII	6,7,12-TMBA	178.5–179.5	7.5	8/8
XXIV	6,8,12-TMBA	138–138.5	15	0/12
XXV	7,8,12-TMBA	126–127	7.5	8/8

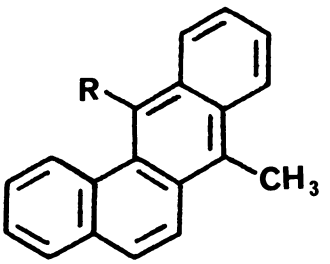

FIG. 2.—The structure of 7-Me-BA. Its derivatives are corticolytic when R = methyl.

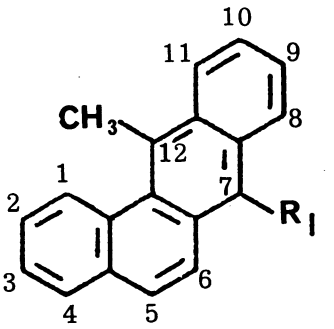

FIG. 3.—The structure of 12-Me-BA. Its derivatives are corticolytic when R_1 = Me; Et; OHMe; formyl; Me-O-Me; 1-OHEt; 2-OHEt; 1-OH-*n*-propyl.

generated in the metabolism of 7,12-DMBA. It would appear that hydroxyalkyl derivatives of 12-Me-BA are the active corticolytic agents.

The entire ring system and the methyl group at C_{12} provide a nonpolar site for attachment in adrenal tissue by charge transfer or hydrophobic bonding. The hydroxy group of the C_7 side chain attached to the *meso*-anthracenic region of the molecule provides another site for polar hydrophilic attachment, presumably by hydrogen bonding.

In sum, these are the attributes in 7-hydroxyalkyl-12-methylbenz(a)anthracenes which combine to endow these molecules with a remarkable ability for the selection and massive destruction of a region of the adrenal: (1) The compounds are fully aromatic. (2) They have close steric resemblance (Fig. 3) to the corticosteroids. (3) They contain both polar and nonpolar groups.

We are indebted to Paul Schurr, the Upjohn Co., Kalamazoo, Michigan, for preparing a lipid emulsion. M. S. Newman, Department of Chemistry, Ohio University, Columbus, generously donated monomethyl derivatives of benz(a)anthracene.

* This investigation was supported by grants from the American Cancer Society and the Jane Coffin Childs Memorial Fund for Medical Research.

† Visiting professor. Present address: Department of Pathology, Kansai Medical School, Osaka, Japan.

‡ The abbreviations used are: BA, benz(a)anthracene; DMBA, dimethylbenz(a)anthracene; DMSO, dimethylsulfoxide; Me-O-Me, methoxymethyl; TMBA, trimethylbenz(a)-anthracene.

[1] Huggins, C., in *Carcinogenesis: A Broad Critique* (Baltimore: Williams and Wilkins, 1966), p. 725.

[2] Huggins, C., and S. Morii, *J. Exptl. Med.*, **114**, 741 (1961).

[3] Pataki, J., and C. Huggins, *Biochem. Pharmacol.*, **16**, 607 (1967).

[4] Currie, A. R., J. Helfenstein, and S. Young, *Lancet*, **2**, 1199 (1962).

[5] Dao, T. L., and Y. Tanaka, *Cancer Res.*, **23**, 1148 (1963).

[6] Huggins, C., and J. Pataki, these PROCEEDINGS, **53**, 791 (1965).

[7] Selye, H., G. Gabbiani, and B. Tuchweber, *Med. Exptl.*, **8**, 74 (1963).

[8] Huggins, C., and T. Sugiyama, *Nature*, **206**, 1310 (1965).

[9] Boyland, E., and P. Sims, *Biochem. J.*, **95**, 780 (1965).

[10] Boyland, E., P. Sims, and C. Huggins, *Nature*, **206**, 1310 (1965).

[11] Schurr, P., *Cancer Res.*, **29**, 258 (1969).