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ADRENAL DESTRUCTION AND CANCER INDUCED BY HYDROXYALKYL DERIVATIVES OF 7,12-DIMETHYLBENZ(a)ANTHRACENE*

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Abstract—Six new derivatives of benz(a)anthracene were found to cause massive and selective destruction of adrenal cortex of young adult female rats.

The molecular requirements of the adrenocorticolytic agents are: (i) they have two and only two substituents; (ii) a methyl group at position 12 was mandatory; (iii) substituents at position 7 possessed an oxygen function or chloromethyl group.

The hydroxyalkyl group diminishes or eliminates carcinogenicity of the parent compound.

A LARGE number of polycyclic aromatic hydrocarbons can evoke cancer, especially in murine species. Two of these compounds, 7,12-dimethylbenz(a)anthracene and 7,8,12-trimethylbenz(a)anthracene exceed all others in carcinogenic activity[1] by about ten times. In biological systems, 7,12-DMBA† exerts remarkable effects which set it apart from other aromatic compounds. These concern (i) its metabolism and (ii) its ability to bring about destruction of adrenal cells. The present work is concerned with the molecular structure of derivatives of benz(a)anthracene in relation to their ability to destroy the adrenal cortex and to their carcinogenicity.

Anthracene,[2] benz(a)anthracene,[3, 4] dibenz(a,h)anthracene[5] and other unsubstituted conjugated aromatic hydrocarbons are metabolized primarily by oxidation at selected centers of unsaturation. Boyland and Sims[6] found that, in contrast to the foregoing, 7,12-DMBA is metabolized primarily by oxidation of its methyl groups to form principally two isomeric monohydroxymethyl derivatives, 7-hydroxymethyl-12-methylbenz(a)anthracene (I) and 12-hydroxymethyl-7-methylbenz(a)anthracene (XVII).

Under simple conditions, a single dose of 7,12-DMBA invariably brings about total and selective destruction[7] of two zones of rat's adrenal cortex while other zones are uninjured. The susceptibility of adrenal to damage by 7,12-DMBA is hormone dependent.[8] The specific damage to the cortex results in adrenal hemorrhage and the striking display of apoplexy in the adrenal glands is reminiscent of red lights in the dark. Previously only three conjugated aromatics have been found to destroy the adrenal; these are 7,12-DMBA and its congeners, perdeuterated 7,12-DMBA (7) and 7-hydroxymethyl-12-methylbenz(a)anthracene.[9] Wheatley *et al.*[10] have presented

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† The following abbreviations are used: BA, benz(a)anthracene; 7,12-DMBA, 7,12-dimethylbenz(a)anthracene.

evidence that 7,12-DMBA itself is not adrenocorticolytic but is metabolized in liver to a substance that causes massive adrenal necrosis.

In the present work, six additional compounds related to benz(a)anthracene were found to destroy adrenal cortex.

METHODS

Chemical

Of thirty compounds which were tested, ten were new and were synthesized in our laboratories. The new compounds are II, III, IV, V, VI, VIII, XIX, XXIII, XXV and XXVI (see Tables 1 and 2). Satisfactory elemental analyses were obtained for each

TABLE 1. ACTIVE COMPOUNDS: THESE DESTROYED SELECTIVELY THE ADRENAL CORTEX

No.	Compound	Minimum effective dose (mg)
I	7-Hydroxymethyl-12-methyl-BA	10
II	7-(1-Hydroxyethyl)-12-methyl-BA	10
III	7-(2-Hydroxyethyl)-12-methyl-BA	15
IV	7-(1-Hydroxypropyl)-12-methyl-BA	50
V	7-Methoxymethyl-12-methyl-BA	50
VI	7-Formyl-12-methyl-BA	50
VII	7,12-Dimethyl-BA	50
VIII	7-Chloromethyl-12-methyl-BA	75

TABLE 2. INACTIVE COMPOUNDS: THESE DID NOT DAMAGE ADRENAL CORTEX

No.	Compound	Maximum dose tested (mg)
IX	7-Methyl-BA	100
X	12-Methyl-BA	100
XI	7-Hydroxymethyl-BA	100
XII	12-Hydroxymethyl-BA	100
XIII	4-Fluoro-7,12-dimethyl-BA	50
XIV	5-Fluoro-7-12-dimethyl-BA	50
XV	8-Fluoro-7,12-dimethyl-BA	50
XVI	11-Fluoro-7,12-dimethyl-BA	50
XVII	12-Hydroxymethyl-7-methyl-BA	100
XVIII	7,12-Dihydroxymethyl-BA	100
XIX	7-Bromomethyl-12-methyl-BA	100
XX	12-Bromomethyl-7-methyl-BA	100
XXI	7,12-Dibromomethyl-BA	100
XXII	7-Methyl-12-ethyl-BA	100
XXIII	7-Methyl-12-(1-hydroxyethyl)-BA	50
XXIV	7-Ethyl-12-methyl-BA	100
XXV	7-Ethyl-12-hydroxymethyl-BA	50
XXVI	7-Hdyroxymethyl-12-ethyl-BA	100
XXVII	7-*n*-Propyl-12-methyl-BA	100
XXVIII	7,12-Diethyl-BA	100
XXIX	7,8,12-Trimethyl-BA	100
XXX	7,12-Dimethyl-7,12-dihydro-BA	100

new compound. Their nuclear magnetic resonance (NMR) spectra are also in complete agreement with the assigned structures.

Biological

Adrenocorticolysis. The experimental animals were female rats of Sprague–Dawley strain, age 45–50 days; weight 140–155 g. At 0 hr, each rat was given, by gastric

tube, a single feeding of the hydrocarbon. At the first test, the maximum quantity of compound dissolved in sesame oil (4 ml) was given but the dose never exceeded 100 mg. If adrenal damage resulted, subsequent doses were smaller than the original one until the minimum effective dose was found.

At 72 hr the animal was decapitated and the adrenals were removed and weighed on a torsion balance. The left adrenal gland was prepared for histology. The right adrenal gland was homogenized in saline and the content of blood pigments in the supernatant was measured in a spectrophotometer; the results were expressed as μg of oxyhemoglobin per gland. An adrenal of the rat which contains more than 125 μg of hemoglobin is the site of hemorrhage. Adrenal apoplexy is hemorrhage visible in the gross. A compound which caused adrenal hemorrhage or apoplexy in every member of a group of five rats is designated an *active compound* and the smallest amount required for this purpose is the *minimum effective dose.*

Carcinogenicity. The experimental animals were male rats, age 21 days, of Long–Evans strain. This strain has great resistance to pulmonary infections making it suitable for long term experiments. Each compound was dissolved in sesame oil to make 0·5 per cent (w/v) solution. There were eight rats per group. Each rat received a 0·5 ml injection in the muscle of each thigh. The rats were observed until sarcomas developed or until 220 days had elapsed after the injection. At necropsy, the presence of oil in the muscle was verified. The diagnosis was confirmed by histology.

RESULTS

1. *Adrenocorticolysis*

All of the active compounds (Table 1) caused a similar sort of lesion in adrenal cortex consisting of total destruction of zona fasciculata and zona reticularis whereas adrenal medulla was uninjured. Usually zona glomerulosa was not damaged but when the gland was very tense with blood, *patches* of destruction of this layer were sometimes observed.

In earlier work[7] 7,12-DMBA (VII) and, more effectively,[9] 7-hydroxymethyl-12-methyl-BA(I) were discovered to be active in destroying the adrenal cortex. This finding was confirmed in the present study and six new active compounds (II–VI; VIII) were identified.

Adrenocorticolytic agents in the benz(a)anthracene series (Fig. 1) have three common characteristics. (i) They have two and only two substituents. (ii) They have

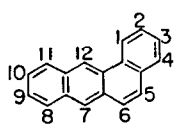

FIG. 1. Benz(a)anthracene.

a methyl group at C^{12}. (iii) They have a hydroxyalkyl or chloroalkyl group at C^7, or a potential hydroxyalkyl group as in compounds V, VI and VII. Enzymatic demethylation of V and reduction of VI in the rat organism would give rise to the highly active compound I. In the case of 7,12-DMBA, the hydroxymethyl substituent at C^7 is formed during metabolism.

i. *The number of substituents.* All of the active compounds (Table 1) had two substituents located, respectively, at C^7 and C^{12}. Compounds with one substituent (IX–XII) were inactive (Table 2). Likewise, compounds with three substituents (XIII–XVI; XXIX) did not destroy adrenal cells.

ii. *Substituent at* C^{12}. 7-Hydroxymethyl-12-methyl-BA (I) is a powerful adrenocorticolytic agent. Its activity is eliminated when its methyl group at C^{12} is replaced by: hydrogen atom (XI); hydroxymethyl (XVIII); or ethyl (XXVI) group.

7,12-DMBA (VII) is metabolized to an active compound, I. The potential of 7,12-DMBA for adrenal destruction is eliminated by replacement of its methyl group at C^{12} by: hydrogen atom (IX); hydroxymethyl (XVII); bromomethyl (XX); ethyl (XXII); 1-hydroxyethyl (XXIII).

iii. *Substituents at* C^7. 12-Methyl-BA (X) is an inactive compound (Table 2) but it is converted to an active corticolytic agent when it possesses at C^7 an additional substituent of a special kind. In order of increasing effectiveness (Table 1) the potentiating substituents introduced into 12-methyl-BA are: hydroxymethyl (I) = 7-(1-hydroxyethyl) (II)$\sim$ 7-(2-hydroxyethyl) (III) $>$ 7-(1-hydroxypropyl) (IV) = 7-methoxymethyl (V) = 7-formyl (VI) $\sim$ 7-chloromethyl (VIII).

Whereas 7-(1-hydroxyethyl)-12-methyl-BA (II) and an isomer, III, are highly effective corticolytic compounds, 7-ethyl-12-methyl-BA (XXIV) is inactive. Likewise, 7-(1-hydroxypropyl)-12-methyl-BA (IV) is an active substance, whereas 7-(*n*-propyl)-12-methyl-BA (XXVII) is inactive.

2. *Carcinogenesis*

Following intramuscular injection, three compounds (VII; XXIV; XXIX) induced sarcomas (Table 3) in high yield in the thigh muscle of rat. The presence of an oxygen

TABLE 3. INDUCTION OF SARCOMAS AFTER INTRAMUSCULAR INJECTION OF HYDROCARBONS*

No.	Compound	No. rats	Rats with sarcoma	Detection of sarcoma (days) Range	Median
VII	7,12-DMBA	8	8/8	67–116	86
XXIX	7,8,12-Trimethyl-BA	8	8/8	64–105	86
XXIV	7-Ethyl-12-methyl-BA	7	7/7	82–120	97
I	7-Hydroxymethyl-12-methyl-BA	8	2/8	139–220	—
II	7-(1-Hydroxyethyl)-12-methyl-BA	8	0	—	—
III	7-(2-Hydroxyethyl)-12-methyl-BA	8	1/8	220	—
IV	7-(1-Hydroxypropyl)-12-methyl-BA	8	0	—	—

* The hydrocarbon, 2·5 mg, dissolved in sesame oil, 0·5 ml, was injected in thigh muscle of male rats of Long–Evans strain at age 21 days and the experiment was terminated 210–230 days thereafter.

function on the alkyl group at C^7 weakened (I; III) or eliminated (II; IV) the potency as a carcinogen of the parent compound which did not possess an oxygen function.

DISCUSSION

In the compounds examined in the present experiments, it was found that an oxygen function on an alkyl group at C^7 conferred adrenocorticolytic activity while causing a decrease of carcinogenicity.

7,12-DMBA has unique properties since it is an extremely powerful carcinogen and at the same time is rather effective in causing adrenal damage. Carcinogenicity is attributed to the unchanged hydrocarbon; adrenocorticolysis is caused by its metabolite which has acquired an oxygen function located at a specific site, C^7.

A mandatory feature of the corticolytic agents was an intact methyl group at position 12 of the benz(a)anthracene molecule. Hydrogen or substituents other than methyl (ethyl; hydroxymethyl; bromomethyl) at C^{12} completely abolish the adrenal-damaging activity of polynuclear aromatic hydrocarbons, indicating that the spatial requirement for that part of the molecule which is delineated by the aromatic ring system must be rather rigid. A difference of 0·7Å in the thickness of this portion of the molecule, going from 7-hydroxymethyl-12-methyl-BA (I) to 7-hydroxymethyl-12-ethyl-BA (XXVI), results in a compound completely devoid of activity. The methyl group either enhances the charge-transfer complexing ability of the ring system or, more probably, takes part in hydrophobic bonding which constitutes a much stronger interaction between molecules than ordinary van der Waals forces.

With regard to the nature of the substituents at C^7, we must differentiate between groups which, *per se*, confer activity upon the molecule (hydroxymethyl; 1-hydroxyethyl; 2-hydroxyethyl; 1-hydroxypropyl) and substituents whose activity depends on their ability to be metabolized in the rat organism (methyl; methoxymethyl; formyl). While the size of the substituent at this site of the molecule is only of secondary importance for adrenocorticolytic activity, the requirements for enzymatic hydroxylation of a C^7-alkyl side chain are more exacting. This is evidenced by the lack of activity of 7-ethyl-12-methyl-BA (XXIV), showing that hydroxylation did not take place in the ethyl group of this compound because both of the possible hydroxylated isomers (II and III) are potent corticolytic agents. There is strong steric hindrance between the methyl groups at C^7 and C^8 of the inactive, 7,8,12-trimethyl-BA (XXIX).

The introduction of fluorine into 7,12-DMBA abolished the activity of this aromatic compound in destroying the adrenal cortex. At the present time, evidence is unavailable to decide if these fluorinated derivatives (XIII, XIV, XV, XVI) are not hydroxylated in the rat organism or if hydroxylated metabolites fail to produce adrenal damage. In either of the two cases, it is rather improbable that this inactivity is due to steric influences of the fluorine atom which is not substantially larger than hydrogen (covalent plus van der Waals radii are 1·99 and 1·50, respectively). Inactivity *vis-à-vis* adrenal must be entirely the consequence of electronic factors due to the electronegativity of fluorine.

The hydroxyl group of the C^7 side chain provides one site of attachment of the molecule in the adrenal tissue, probably by hydrogen bonding. The whole ring system provides the other site for charge-transfer, or hydrophobic bonding.

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