Molecular Structures of the Chemical Carcinogens 7-Chloromethylbenz[a]anthracene and 7-Chloromethyl-12-methylbenz[a]anthracene

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SUMMARY

The three-dimensional structures of two carcinogens, 7-chloromethyl-12-methylbenz[a]anthracene and 7-chloromethylbenz[a]anthracene, have been determined by X-ray crystallographic techniques. Both compounds are carcinogenic and are believed to act by alkylating DNA. However, the first has a nonplanar ring system, whereas the second has a planar ring system. The nonplanarity of 7-chloromethyl-12-methylbenz[a]anthracene results from steric hindrance between a hydrogen atom of the 12-methyl group and a hydrogen atom on the [a] ring. This molecule cannot be made planar unless the 12-methyl group or the [a] ring is removed.

It is concluded that the carcinogenic activity of these compounds does not correlate with planarity of the ring system. This implies that, if DNA is the critical target of attack by these carcinogens, complete intercalation of the aromatic ring system of the carcinogen between the bases of DNA is not a likely mechanism of carcinogenic action in this system of compounds. The results presented here and those of others are more consistent with a model for a common interaction of the carcinogens 7-chloromethyl-12-methylbenz[a]anthracene and 7-chloromethylbenz[a]anthracene with DNA, in which they alkylate the bases of DNA and then lie with their long axes approximately parallel to the helix axis, probably in the major groove.

INTRODUCTION

An ultimate aim of those who study chemical carcinogenesis is to be able to describe, in 3-dimensional terms, the details of the interaction of the carcinogen with the critical target. In this way we will be able to characterize those features that make a chemical carcinogenic. There is not much detailed physical information available to date on the structures of chemical carcinogens or on the correlation of 3-dimensional structure with activity. Since the mode of interaction of these carcinogens with the critical target may depend on chemical reactivities at certain sites in the molecules, or on the geometrical configurations of the carcinogens, or both, it is important to study the 3-dimensional structures of such compounds in detail. We report here the crystal structures of 2 carcinogenic chloromethyl alkylating agents related to DMBA,1,2 I. They are 7-CICH2-MBA, II, and 7-CICH2-BA, III; the formulae are shown in Chart 1. The study was undertaken in order to investigate whether there is a conformational relationship among these 3 analogous carcinogenic compounds. Their 3-dimensional structures, so derived, were examined in the light of hypotheses that their carcinogenic activities result from alkylation of DNA, from intercalation in DNA, or from both types of effects.

The mechanism of chemical carcinogenesis by the polycyclic aromatic hydrocarbon DMBA (I) has been extensively investigated. Dipple et al. (7) suggested that a carbonium ion, Ar-CH=+, might be a reactive intermediate in such carcinogenesis. 7-Hydroxymethyl-12-methylbenz[a]anthracene, a metabolite of DMBA (4, 5, 10), was found to induce cancer in rats and mice. Because this metabolite might have resulted from a carbonium ion from DMBA, some bromo- and chloromethyl derivatives of benz[a]anthracene were prepared and studied (5, 8, 9) since, it was reasoned, these compounds might readily yield carbonium ions. The compounds, when prepared, were found to show carcinogenic (8, 9, 10, 22), antitumor (21), and mutagenic activities (18). They are inhibitors of RNA as well as of DNA synthesis (5), and their binding to DNA in vitro and in vivo has been investigated (2, 25, 27). The general result from these studies is that 7-BrCH2-BA is less carcinogenic than the compound with an additional 12-methyl group (8, 9), whereas both chloromethyl compounds (II and III) are almost equally carcinogenic (22).

MATERIALS AND METHODS

Crystals of 7-CICH2-MBA (II)3 and 7-CICH2-BA (III)4 were prepared by Dr. R. M. Peck of this Institute. The experimental X-ray work, with pertinent results, is reported in Table 1. Details will be published elsewhere.2,4 The structures were refined to conventional R values of 0.045 and 0.063 for I and III, respectively, for which R value = Σ[|Fo| - |Fc|]/Σ|Fo|.

1 Research supported by Grants CA-10925, CA-06927, and RR-05539 from the NIH, USPHS, by Grant AG-370 from the National Science Foundation, and by an appropriation from the Commonwealth of Pennsylvania.
2 The abbreviations used are: DMBA, 7,12-dimethylbenz[a]anthracene; 7-BrCH2-MBA, 7-bromomethyl-12-methylbenz[a]anthracene; 7-BrCH2-BA, 7-bromomethylbenz[a]anthracene; 7-CICH2-MBA, 7-chloromethyl-12-methylbenz[a]anthracene; 7-CICH2-BA, 7-chloromethylbenz[a]anthracene; ApU, adenylyl-3’5’-uridine; UpA, undylyl-3’5’-adenine.
3 H. L. Carrell, manuscript in preparation.
4 D. E. Zacharias, manuscript in preparation.
where $F_o$ is an observed structure factor and $F_c$ is a calculated structure factor. All atoms, including hydrogen atoms, were located, and their positions were refined. We chose to work on the chloromethyl compounds because chlorine, with a lower atomic number than bromine, does not dominate the X-ray scattering to as great a degree, so that atomic positions, including those of hydrogen atoms, could therefore be determined with higher precision.

RESULTS

Comparison of Bond Lengths and Bond Angles. A comparison of interatomic distances in molecules of 7-CH$_2$-MBA (II), and 7-CH$_2$-BA (III), determined in these crystallographic studies, is given in Chart 2. Differences in bond lengths that are possibly significant (greater than 5o, where o is the estimated standard deviation of a measurement) are indicated by heavy lines in this chart.

As shown in Table 1, II crystallizes with 2 nonidentically packed molecules (designated IIA and IIB) in the crystal. Therefore, molecular parameters for each IIA and IIB are determined independently. The differences in bond lengths between Molecules IIA and IIB all lie within 2.7o, so that averaged values are given in Chart 2. The exception is 2 bonds in the [a] ring where each value is given in Chart 2 (marked by asterisks). This is a region in each molecule where the thermal motion is high (that is, much vibration is possible); therefore, positions of atoms are less accurately determined. It is also the area where Molecules IIA and IIB interact with each other (see below).

Large differences in bond lengths between Molecules II (average) and III (Chart 2) occur mainly at the junction of the C and D rings, an area affected by distortion due to the 12-methyl group. The shortest bond, a double bond, lies in the K-region for each compound, i.e., the area in the aromatic molecule that behaves most like an olefinic double bond. However, it can be seen in Chart 2 that bond lengths around the K-region are similar in both II and III, so that 12-methylation does not have a significant effect on the K-region.

Since accurate bond lengths, which are dependent on the multiplicities of the bonds, have been determined for both Molecules II and III, an attempt was then made to describe the areas of highest $\pi$-electron density and, hence, of reactivity. This was done by computing $\pi$ bond orders for each bond in the compounds. These were derived assuming a $\pi$ bond order of zero for a bond of length 1.54 Å (diamond), 0.667 for a bond of length 1.397 Å (benzene), and 1.00 for a bond of length 1.334 Å (ethylene) (26). In both II and III, the highest $\pi$ bond order is, as expected, that in the K-region.

Free valences or unsaturation indices for each atom in the molecules were then calculated from the formula:

$$[1.732 - \Sigma (\pi \text{ bond orders}) = \text{free valence}]$$

as described by Pullman and Pullman (26). The summation ($\Sigma$) was made over all bonds connected to the atom for which the free valence is being determined. Values of free valences are very sensitive to the curve chosen to describe bond order. Such curves vary principally in the value chosen for a "single bond" in an aromatic ring system [that for diamond (1.54 Å) was used here]. In general, atoms in such conjugated systems with high free valences would be expected to be most susceptible to attack [electrophilic, nucleophilic, or free radical (26)].

Values for free valences calculated in this way are listed in Table 2. As shown in this table by asterisks, the change in free valence between Molecules II and III occurs mainly in the area of nonplanarity (Positions a to j).

Newman et al. (19, 20), who have studied this system extensively, suggest that the reactive intermediate in the case of DMBA may be a compound with a keto group at position 5 and a methylene group at position 4, possibly formed from an epoxide intermediate. In each of the compounds I, II, and III, it can be seen in Chart 3 that the free valence at Position 5 is less than that at Position 4. This suggests that all 3 compounds may behave in a similar manner on reaction in this area of the molecule. In all 3 compounds (I, II, and III) the highest free valence is at Position 12.

It is concluded from a study of interatomic distances in II and III that the K regions of these 2 compounds are similar and that the major differences occur near the junction of Rings C and D (Chart 4). This is the area that might be expected to be most affected by the overcrowding caused by the 12-methyl group in II.

Planarity of the Ring Systems. The aromatic ring system of II, like that of DMBA (I) (12, 29), and the K-region oxide of DMBA (11), is markedly nonplanar. There are angles of 21°.
Table 1
Crystal and activity data on II and III

<table>
<thead>
<tr>
<th></th>
<th>7-CICH&lt;sub&gt;2&lt;/sub&gt;-MBA (II)</th>
<th>7-CICH&lt;sub&gt;2&lt;/sub&gt;-BA (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;Cl</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;Cl</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Monoclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>P&lt;sub&gt;2&lt;/sub&gt;/c</td>
<td>P&lt;sub&gt;2&lt;/sub&gt;/n</td>
</tr>
<tr>
<td><strong>Cell dimensions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>20.457 (4) Å</td>
<td>14.381 (7)&lt;sup&gt;α&lt;/sup&gt; Å</td>
</tr>
<tr>
<td>b</td>
<td>11.474 (2) Å</td>
<td>4.228 (2) Å</td>
</tr>
<tr>
<td>c</td>
<td>13.034 (2) Å</td>
<td>22.141 (7) Å</td>
</tr>
<tr>
<td>β</td>
<td>108.91 (1)&lt;sup&gt;α&lt;/sup&gt; o</td>
<td>95.87 (5)&lt;sup&gt;α&lt;/sup&gt; o</td>
</tr>
<tr>
<td>V</td>
<td>2894 (1) Å&lt;sup&gt;α&lt;/sup&gt;</td>
<td>1339 (2) Å&lt;sup&gt;α&lt;/sup&gt;</td>
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<td><strong>Cell contents</strong></td>
<td>8 molecules (2 per asymmetric unit)</td>
<td>4 molecules (1 per asymmetric unit)</td>
</tr>
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<td><strong>Observed density</strong></td>
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<td>1.37 g cm&lt;sup&gt;-3&lt;/sup&gt;</td>
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<tr>
<td><strong>Calculated density</strong></td>
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<td>1.38 g cm&lt;sup&gt;-3&lt;/sup&gt;</td>
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<tr>
<td><strong>No. of independent intensity data collected</strong></td>
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<td>2490</td>
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<tr>
<td><strong>No. of reflections below threshold value</strong></td>
<td>1907</td>
<td>773</td>
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<td><strong>Radiation used</strong></td>
<td>CuKα (1.5418 Å)</td>
<td>CuKα (1.5418 Å)</td>
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<td>Direct methods</td>
<td>Direct methods</td>
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<td><strong>Final R value</strong></td>
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<td>0.063</td>
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<td>Yes</td>
</tr>
<tr>
<td><strong>Estimated standard deviations in C–O or C–C bond lengths</strong></td>
<td>0.004 Å</td>
<td>0.005 Å</td>
</tr>
<tr>
<td><strong>Angle between outermost rings</strong></td>
<td>20.8, 21.8°</td>
<td>1.5°</td>
</tr>
<tr>
<td><strong>Relative biological activity of the corresponding bromo compounds (8, 9)</strong></td>
<td>Products with DNA more light sensitive. Higher specificity for attack of adenine than guanine residues in DNA</td>
<td>Products with DNA less light sensitive. Lower ratio adenine:guanine substituted in DNA</td>
</tr>
<tr>
<td><strong>Conclusions by Rayman and Dipple (27) for the bromo compounds</strong></td>
<td>More carcinogenic</td>
<td>Less carcinogenic</td>
</tr>
</tbody>
</table>

<sup>α</sup> Values in parentheses are estimated standard deviations with respect to the last digit given.

24°, and 35°, respectively, between the 2 outermost rings (Chart 4, A and D), of these 3 compounds (Table 3). In contrast, the ring system of III is almost completely planar (excluding the –CH<sub>2</sub>Cl group).

The nonplanarity of the ring system of II is an inherent property of the molecule that cannot be changed (unless the 12-methyl group or the angular ring is removed), and it is found in other 12-methylbenz[a]anthracene derivatives (13). The nonplanarity results from steric hindrance between a hydrogen atom of the 12-methyl group and a hydrogen atom on the angular ring. This is illustrated diagrammatically in Chart 3. The nonplanarity in DMBA (I), reported in its crystal structure (12, 29), results from an identical interaction. Nonplanarity in I, II, and the oxide of DMBA confers optical activity on these compounds (since mirror images of the molecules cannot be superimposed on the original molecule), and it is possible that one optical isomer of a given compound may be biologically more active than the other.

If the 2 compounds II and III have similar conformations of their ring systems when they interact with a biological macromolecule, this conformation must be nonplanar. This is possible since III can buckle, whereas it is not possible for II to have a flat ring system.

Packing in the Unit Cell. A striking difference in the structures of these compounds is that III crystallizes with only 1 molecule in the asymmetric unit, whereas II crystallizes with 2 nonidentically packed molecules (designated IIA and IIB) in the asymmetric unit. There seems to be no specifically strong interaction between these 2 nonidentically packed molecules in the overall crystal unit cell. Excluding interactions involving chlorine atoms, the closest interactions between Molecules IIA and IIB are van der Waals-type contacts between the [a] rings shown in Chart 4. This is a "herring-bone"-type packing. That is, the aromatic ring systems do not lie in planes parallel to each other, but inclined to each other. As a result one (or more) hydrogen atom(s) of one molecule interact with the π-electron system of the other molecule. This is characteristic of many aromatic compounds, e.g., benzene.

The tendency for carcinogens to crystallize with more than 1 molecule in the asymmetric unit has been noted before (17). It may reflect the awkward shapes of such molecules, making efficient packing possible only with 2
independent units. Alternatively, the interaction of a hydrogen atom of one molecule with the π-electron system of another may be a predominantly stable interaction. Mason (17) called the number of molecules in the asymmetric unit an "aggregation factor" which he attempted to correlate with carcinogenic activity. However, no direct correlation has been found.

The molecules can also pack in planes parallel to each other. The stacking perpendicular to the general direction of the planes of the ring systems is illustrated for each compound in Chart 5. The areas of stacking involve only the more planar portions of the molecules. Molecules of II (Chart 5a) pack as head-to-tail "dimers" between like molecules, i.e., IIA packs with another IIA molecule, and IIB packs with another IIB molecule. As noted earlier, IIA packs with IIB in a "herring-bone" fashion. The more planar portion of II (on the side of chloromethyl substitution) overlaps slightly (as shown by the shading) in planes separated by 3.55 Å. The chlorine atoms point away from the center of the dimer. The area of maximum nonplanarity does not take part in the overlap of the dimers. Molecules of III (Chart 5b) pack in a stepwise manner throughout the crystal in planes 3.55 Å apart so that both sides of this flatter molecule are involved in stacking.

In both types of molecules the K-region double bond of one molecule lies above a nonlocalized ring bond (i.e., a more aromatic type of bond) of another molecule. The area of the molecules involved in vertical stacking (shaded in Chart 5, a and b) is less than that found in crystals of the intercalator 9-aminoacridine (Chart 5c) (31) as well as in crystals of the complex of 9-aminoacridine with ApU (a model for intercalation of a polycyclic molecule in DNA (30) (Chart 5d). It can be seen in Chart 5 that, in its interaction with ApU, there is much overlap of 9-aminoacridine with the bases and with the hydrogen bond system between the bases.

Both molecules, then, show some tendency to stack in parallel planes ~3.5 Å apart, although only the planar portions of the molecules are involved in this stacking. However, the extent of stacking is less than that found for some known intercalators.

Conformations of Molecules. The shapes of molecules of II and of III are shown in Chart 6. These are views (1, 3) in directions perpendicular to and parallel to the best plane through the [a] ring (D ring, chosen arbitrarily). Spheres with van der Waals radii for each atom are stippled over the bond diagrams. Views onto the molecular planes are given in Chart 6, a and b. These indicate the large hydrophobic area that is expected to lie in the confines of the bases of the DNA helix if intercalation occurs (6). Side views of Molecule II in Chart 6, c and e, show the extent of buckling of the ring systems compared with Molecule III in Chart 6, d and f. It is shown in Chart 6e that the buckling of molecules of II causes them to be wedge shaped (also shown in Chart 6c). The vertical lines in Chart 6, e and f, indicate the width of the space that would be available for intercalation between the bases of DNA.
Chart 3. Diagram illustrating the steric hindrance between the 12-methyl group and the hydrogen atom of the [a] ring in II. This steric hindrance does not occur for III.

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Refs.</th>
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<tbody>
<tr>
<td>DMBA</td>
<td>(Ref. 12 and 28)</td>
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<tr>
<td>7-CICH2-MBA</td>
<td>(this work)</td>
</tr>
<tr>
<td>7-CICH2-BA</td>
<td>(this work)</td>
</tr>
<tr>
<td>K-region oxide of DMBA</td>
<td>(Ref. 11)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angle</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tbody>
<tr>
<td>A:B</td>
<td>5.0</td>
<td>3.8</td>
<td>3.9</td>
<td>2.1</td>
</tr>
<tr>
<td>A:C</td>
<td>13.2</td>
<td>11.3</td>
<td>12.3</td>
<td>2.0</td>
</tr>
<tr>
<td>A:D</td>
<td>24.0</td>
<td>20.8</td>
<td>21.8</td>
<td>1.6</td>
</tr>
<tr>
<td>B:C</td>
<td>10.8</td>
<td>8.1</td>
<td>9.0</td>
<td>0.7</td>
</tr>
<tr>
<td>B:D</td>
<td>21.2</td>
<td>17.5</td>
<td>18.4</td>
<td>1.0</td>
</tr>
<tr>
<td>C:D</td>
<td>10.8</td>
<td>9.6</td>
<td>9.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Chart 4. The only interactions between molecules II A and II B. These involve packing of the D rings of each molecule against each other.

Thus, molecules of II could not slip easily between base pairs in DNA without causing considerable distortion of the helix axis. No such problem exists for molecules of III. This is illustrated diagrammatically in Chart 7a where it is shown that, if they are inserted between the bases of DNA, II and III have different effects on the direction of the DNA helix axis.

DISCUSSION

It is not known whether the critical target in carcinogenesis is a protein, a nucleic acid, or another type of molecule. However, since II and III are both mutagens and carcinogens, it is of interest to consider the effects of the interaction of these compounds with DNA.

Importance of Planarity in Carcinogenesis in Benzo[a]anthracene Derivatives. Of the 2 carcinogens studied here, one has a planar ring system whereas the other does not. In the bromomethylbenzo[a]anthracenes, which presumably have similar structures, the reportedly more potent species has a markedly nonplanar ring system. We conclude that planarity of the aromatic system in haloalkylbenzo[a]anthracenes is not a requirement for carcinogenicity. Further, ring system planarity may not be important in antitumor or mutagenic activity, since both chloromethylbenzo[a]anthracenes (II and III) are active.

Importance of Intercalation in Carcinogenesis by Benzo[a]anthracene Derivatives. Intercalation may be defined as the complete envelopment of a flat hydrophobic molecule within the hydrophobic confines of a DNA helix, without displacement or distortion of the helix direction (6). Detailed studies (15) of the binding of benzo(a)pyrene to DNA have led to a corroboration of the intercalation model, and they showed that the more compact ring systems bind more strongly. Craig and Isenberg (6) showed that, if a polycyclic aromatic hydrocarbon was too large in area to lie within the bases of DNA, protected from the aqueous environment, binding and, hence, intercalation, could not occur. From structural studies on intercalation, in the cases of a 9-aminoacridine:ApU complex (30) and of an ethidium:5-iodo...
UpA complex (32), it is known that the intercalating agent must be reasonably flat. We therefore must conclude that II (7-ClCH₂-MBA) cannot intercalate well in DNA. The situations for insertion of the 2 compounds (II and III) between the bases of DNA are shown in Chart 7a. The 2 compounds have different effects.

Since both Compounds II and III are carcinogenic and yet one of them, II, cannot intercalate well in DNA, we conclude that these compounds do not exert their carcinogenic activity simply by intercalation between the bases of DNA. This must be true unless there is conversion of II in vivo to a compound lacking either the 12-methyl group or the [a] ring so that the ring system becomes planar. Since DMBA is so much more active than the compound lacking a 12-methyl group and since anthracene derivatives are less carcinogenic than benz[a]anthracene derivatives, we conclude that this metabolic demethylation or ring opening is unlikely. Otherwise, the simpler compound might be expected to be more active, whereas, in fact, the reverse is the case.

It is not implied that intercalation of III, for example, does not occur but simply that this form of interaction is not important in carcinogenesis. An interaction of a carcinogen with a protein receptor involved in some way with DNA [such as a steroid receptor (16) or a polymerase] probably has a much lower requirement for planarity of the ring system than does intercalation in a double helical nucleic acid.

**Importance of Alkylation of DNA by Benz[a]anthracene Derivatives.** The compounds that have been studied here are alkylating agents, and it is interesting to consider the consequences of the alkylation of DNA by II and III, since these compounds have also been shown to be mutagens, in which case they almost certainly interact in some way with DNA. It has been shown that amino groups on the bases of DNA are major sites of attack (14, 27, 28). The C—Cl bond of the alkylating agents lies approximately at an angle of 109.5° to the plane of the ring system, and there is no flexibility in this side chain except for rotation about the carbon-carbon bond adjacent to the carbon-chlorine bond (see Charts 3 and 6, e and f).

In this model we assume that epoxidation is unimportant and that the simple halomethyl group alkylates DNA. When such alkylation occurs the chlorine atom is replaced by DNA. As a result, as shown in Chart 7b, the covalently bound carcinogen cannot lie in the plane of the bases (for steric reasons) but must also lie at an angle of approximately 109.5° to the planes of the bases.

Studies with an endonuclease II from *Escherichia coli* (14) showed that this enzyme excises \( N^\circ-(12\text{-methylbenz[a]}\text{anthracenyl}-7\)-methyl)adenine and \( N^\circ-(12\text{-methylbenz[a]}\text{anthracenyl}-7\)-methyl)guanine, particularly the adenine derivative, when DNA is alkylated with 7-BrCH₂-MBA. Fluorescence studies of the interaction of 7-BrCH₂-BA with DNA (23, 24) have indicated that alkylation of the bases of DNA occurs without distortion or denaturation of the secondary structure of DNA. This suggests that a base displacement mechanism, postulated for the interaction with DNA with certain carcinogens (35), does not occur in the benz[a]anthracene system. The red shift in the absorption spectrum on interaction of the carcinogen with DNA was believed to indicate an arrangement of aromatic rings parallel to the helix axis, i.e., perpendicular to the base pairs. This model is in agreement with our reasoning above.

**General Conclusion.** Our structural data indicate that intercalation of the 2 compounds between the bases of DNA is not a likely mechanism of carcinogenic action. Since alkylation and intercalation are not simultaneously possible for sterical reasons and since partial insertion of the carcinogens between the bases of DNA will cause a large hydrophobic area of the carcinogen to protrude beyond the confines of the double helix into the aqueous environment [which Craig and Isenberg thought unlikely (6)], we conclude that the most plausible common mechanism for ac-

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**Chart 5.** Views of molecules perpendicular to the plane through the ring system, showing, by shading, the overlap with molecules in parallel planes approximately 3.5 Å apart. a, Molecule II, “head-to-tail” dimers. The upper molecule is drawn with heavy lines. The chlorine atom is indicated by an open circle. b, Molecule III, “head-to-head” stacking throughout the crystal. The central molecule in this group of 3 molecules is drawn with heavy lines. c, 9-aminoacridine (31), showing extensive overlap for this intercalator. The central molecule is drawn with heavy lines. Nitrogen atoms are indicated by open circles. d, 9-aminoacridine-ApU complex (30), showing the intercalation of 9-aminoacridine between the bases. Note how the polycyclic molecule lies between hydrogen-bonded base pairs.

**Chart 6.** Views of the molecules with respect to the best plane through Ring D. These diagrams were drawn with the program STIPL (1). Rings A, B, C, and D are marked. The thickness of an aromatic ring system is shown (e and f). Molecule II (7-ClCH₂-MBA) is shown in a, c, and e, and an analogous view of Molecule III (7-ClCH₂-BA) is shown in b, d, and f, respectively.
tion of the 2 compounds on DNA is alkylation of the bases. As a result, the carcinogen will lie in a groove of DNA, presumably the major groove, as shown diagrammatically in Chart 7, b and c. This model, although relevant to an interaction with DNA and, possibly, relevant to mutagenesis, is, of course, relevant to carcinogenesis only if it can be shown that DNA is the critical target of attack of the carcinogen. Similar models are being considered for the interaction of steroids with DNA (33, 34). In the model just described (which is similar to that proposed by Pochon et al. (23, 24)), planarity of the aromatic ring system is not essential, although the positioning of the 12-methyl group may be important. The carcinogen, in this model, is inclined at an angle to the bases of DNA, but it does not perturb the double helical DNA structure. It may, however, interfere substantially with some base-recognition system.

ACKNOWLEDGMENTS

We particularly wish to thank Dr. Richard M. Peck of this Institute for providing us with the crystals, and we thank Dr. Richard M. Peck, Dr. Sam Sorof, and Dr. Ronald Harvey for many helpful discussions.

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Molecular Structures of the Chemical Carcinogens
7-Chloromethylbenz[a]anthracene and 7-Chloromethyl-12-methylbenz[a]anthracene

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