Intercalation of 7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Enantiomeric Isomers with Dinucleoside Dimers: A Basis for Alkylation of the 2-Amino Group in Guanine

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Abstract □ The minimum-energy intercalation-complex geometries of the (±)enantiomers of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (I) with two dinucleoside dimers were determined. The purpose of these calculations was to see if I could intercalate into DNA in such a way that the observed alkylation of the 2-amino group of guanine could occur subsequent to intercalation. For both dinucleoside dimer sequences, it was found that the (+)-(9α,10α) isomer could form a stable intercalation complex in which the orientation and distance of the epoxide of I to the 2-amino group of guanine was close to the calculated critical transition-state geometry for the alkylation reaction. The (−)enantiomers can intercalate, but not in a manner close to the transition-state geometry necessary for the alkylation of the 2-amino group of guanine.

Keyphrases □ 7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene—enantiomeric isomers, alkylation of dinucleoside dimers, intercalation-complex geometry and energetics □ Dinucleoside dimers—alkylation by enantiomeric isomers of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, intercalation-complex geometry and energetics □ Intercalation—of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene into dinucleoside dimers, energetics, complex geometry

The previous paper (1) reported that a sterically acceptable transition-state geometry for the alkylation of the amino group of guanine by 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (II) and 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (III) could involve intercalation. Also, a noncovalent complex formation between benzo[a]pyrene and calf thymus DNA has been observed recently (2). This interaction is postulated to involve intercalation of the aromatic hydrocarbon between adjacent base pairs. An equivalent type of intermolecular geometry could also be expected for single-stranded DNA in which the isomeric forms of I are associated with a nucleic acid base or a pair of adjacent bases. This is an important consideration since the total extent of the alkylation of the 2-amino group of guanine by (±)II is observed to be the same for both single- and double-stranded DNA (3). In addition, (+)III forms the major adduct (60–80% of total adducts) with the amino group of guanine (3).

These findings have prompted the exploration of the possibility that the intercalation process might occur as a prior step to alkylation of the 2-amino group of guanine by I in double-stranded DNA. The energetics of the intercalation of (±)II and (±)III (Fig. 1) with dinucleoside dimer sequences was investigated using molecular mechanics energy calculations (4). The results of these calculations are reported herein.

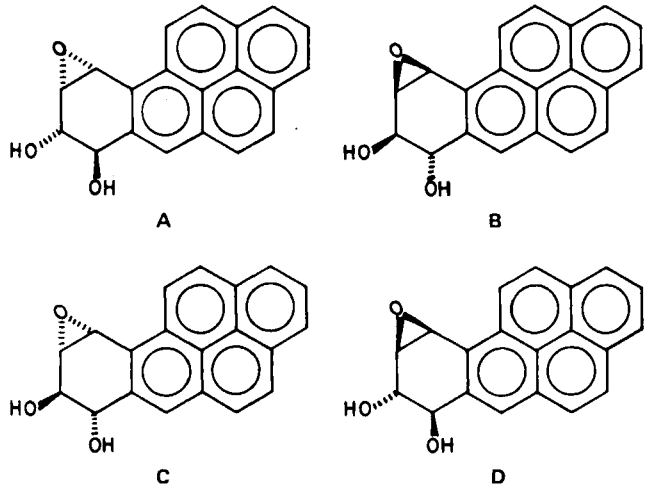

Figure 1—*The enantiomeric isomers of I. Key: (A) (+)II; (B) (−)II; (C) (+)III; (D) (−)III.*

EXPERIMENTAL

The overall procedure for these calculations is identical to that employed previously for the intercalation investigation of doxorubicin (5); a summary of the procedure follows. The DNA structure was represented by a dinucleoside dimer containing guanine. The same geometries used in the doxorubicin study were assigned to the dinucleoside dimers. There are seven unique ways of interacting I with guanine bases in dinucleoside dimers (Fig. 2). One sequence, has two possible 2-amino alkylation sites. Thus eight intermolecular complexes should be studied. However, if

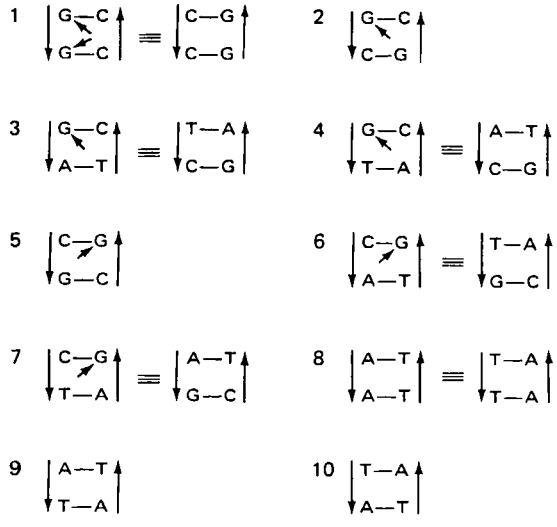

Figure 2—*The I-guanine interaction sites (indicated by the arrows) for dinucleoside dimer base sequences.*

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Table I—Intercalation Properties of Isomers of I with Dinucleoside Dimers

Dinucleoside Dimer	Isomer	Intercalation Energy, kcal/moles/complex	Mode[b]	Position of I[a] r, Å	θ_1, °	θ_2, °	ϕ_1, °	ϕ_2, °	ϕ_3, °
↓(C—G)↑	(+)II	−88.27	d–e	3.7	126	118	97	161	160
		−84.00	a–b	7.6	52	112	140	13	−125
	(−)II	−89.02	e–f	4.2	97	82	80	−77	156
		−88.55	a	5.5	42	126	113	24	−162
	(+)III	−85.94	c	6.8	90	142	143	81	−178
		−85.07	d–e	3.1	123	110	90	168	−148
	(−)III	−88.65	e–f	4.2	100	81	79	−79	151
		−88.12	d	4.8	107	79	126	−179	125
↓(T—A)↑	(+)II	−89.70	d–e	3.7	104	115	−56	80	175
		−82.58	f	4.2	94	78	−123	−167	−154
	(−)II	−88.79	a	5.2	71	126	−138	−69	−168
		−83.54	d	4.0	63	86	−65	126	145
	(+)III	−86.90	d–e	3.1	111	105	−63	84	176
		−84.43	f	4.2	97	80	−118	−171	−159
	(−)III	−89.00	d	3.9	62	86	−65	126	146
		−80.87	a	5.6	55	139	−145	−48	−173

[a] The transition-state geometric variables. The geometry for I–NH_3 is r = 2.0 Å, θ_1 = 110°, θ_2 = 80°, ϕ_1 = 100°, ϕ_3 = 0 or 180°, with ϕ_2 variable. [b] As defined by the intercalation complex geometries in Fig. 5. The designation i–j indicates a reaction-state geometry between two of the designated complexes.

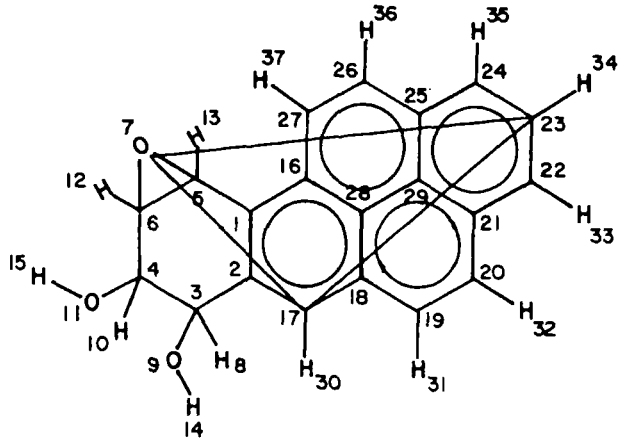

Figure 3—*Structure of I (indicating the internal atomic numbering used) with the descriptive intercalation triangle superimposed.*

detailed sequence specificity is set aside, there are only two cases to consider for the position of an isomer of I. One case involves an isomer of I interacting with guanine from the 3′-side of the base-pair plane, the other case an interaction from the 5′-side of the base-pair plane. Only these two possibilities were considered in this study. Two dinucleoside sequences were employed in the intercalation analyses:

↓(C—G)↑ (5′ side) IV ↓(T—A)↑ (3′ side) V

A specialized version of the MASS (Molecular Assembly Software System) option in the CHEMLAB software package (6, 7) was used to carry out the molecular mechanics calculations. The molecular and electronic structures of (±)II and (±)III used in the study are based on previous calculations (1, 8). The valence geometries of these molecules were held rigid in the intercalation calculations. The structure of I with a superimposition of the descriptive intercalation triangle, involving atoms O(7), C(17), and C(23) as vertices, is shown in Fig. 3. The descriptive intercalation triangle makes it possible to visualize easily the orientation of an isomer of I relative to a dinucleoside dimer. The atomic coordinates of II and V have been given previously (1).

The complexing energy was minimized as a function of the six intermolecular degrees of freedom defined in Fig. 4. Each enantiomer of II and III was placed such that the epoxide ring was above or below the guanine base. Six selected starting positions were chosen in each energy minimization (Fig. 5). It is seen from Fig. 5 that both major and minor groove intercalation were considered.

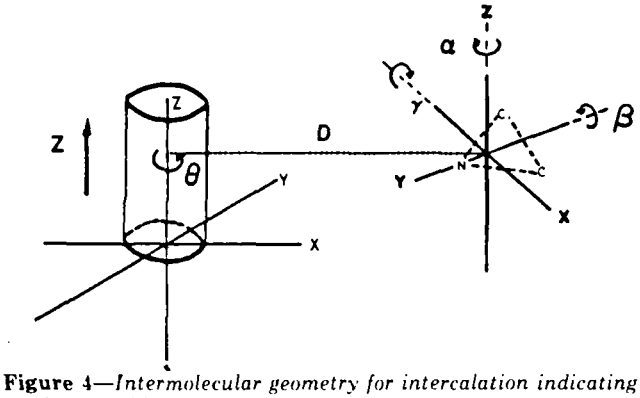

Figure 4—*Intermolecular geometry for intercalation indicating the six degrees of freedom (from Ref. 7).*

RESULTS

The minimum energy complexes for both dinucleoside dimer sequences are reported in Table I. The preferred intercalation structures are vir-

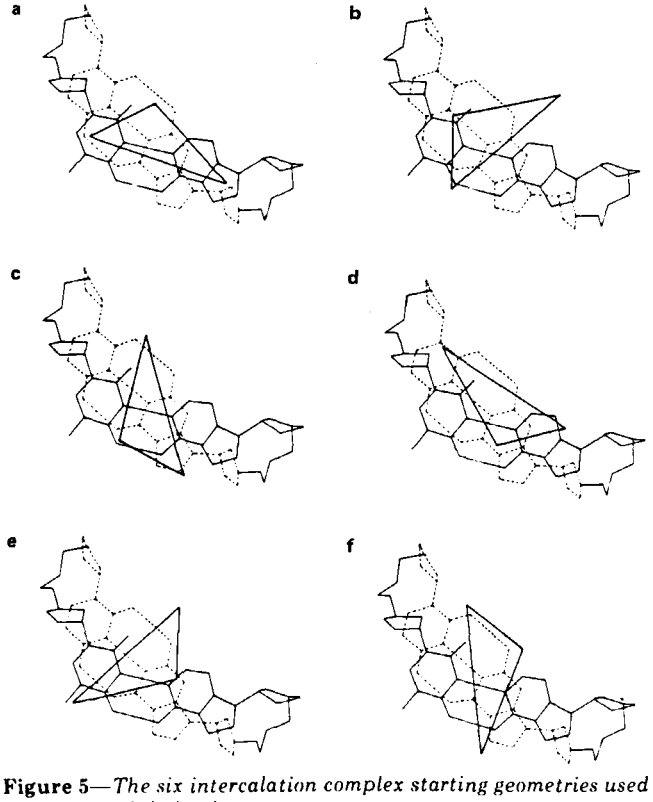

Figure 5—*The six intercalation complex starting geometries used in the energy minimizations.*

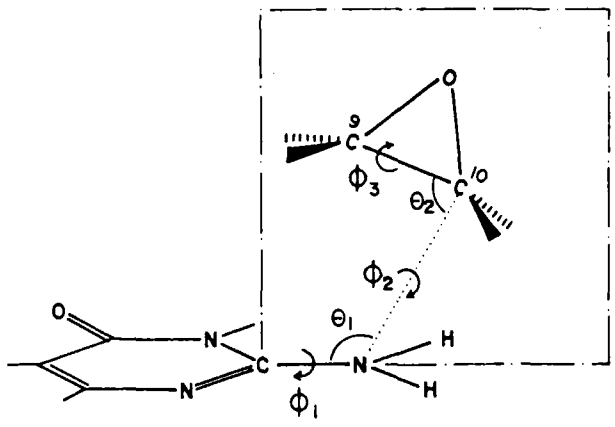

Figure 6—*The I-NH_2-guanine transition-state geometry (from Ref. 1).*

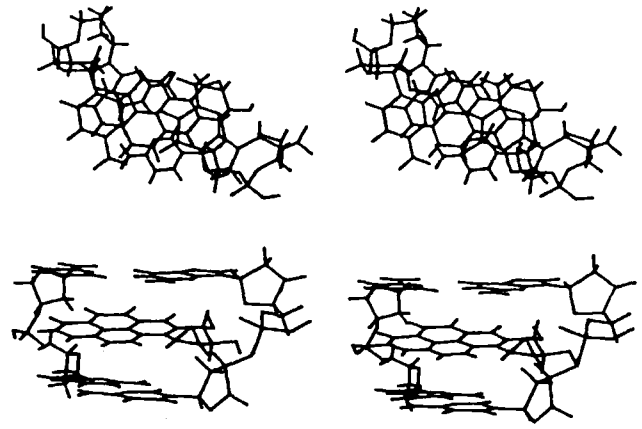

Figure 7—*Stereo-stick models of the (+)II–IV intercalation complex in which the epoxide group of II is close to the 2-amino group of guanine and in the transition-state orientation. The top figures are looking down at the base-pair planes; the bottom figures are looking into the dinucleoside dimer.*

tually independent of the approach of I from either the major or minor groove. The position of each enantiomeric isomer is reported in terms of the geometric variables of the transition state (1, 8). Many of the complex energies are close to one another (~1 kcal/mole). Consequently, it is not possible to identify the most stable intermolecular complex as a function of isomer and/or base sequence. The uncertainty in these computed energies is probably larger than several of the corresponding differences, thus negating such a structural assignment. However, the important observation is that intercalation geometries predisposed for NH_2-alkylation are among the most stable intermolecular structures.

The minimum-energy transition-state geometry has been computed for isomers of I with ammonia as $r = 2.0$ Å, $\theta_1 = 110°$, $\theta_2 = 80°$, $\phi_1 = 100°$, and $\phi_3 = 0$ or 180°, with ϕ_2 variable (8). This model system (Fig. 6) should be a reasonable approximation for I alkylating the 2-amino group of guanine and has been used to evaluate the reactive potential of the various intermolecular complexes.

The complexing energies show a moderate sensitivity to both choice of isomer and base-pair sequence. One consistent observation is that (+)III forms the least stable intercalation complex with the two dinucleoside dimers. The (+)III intercalation complex also possesses the minimum C(10)—NH_2 interaction distance of all complex structures. The second smallest interaction distance occurs in the (+)II–dinucleoside dimer complexes. However, (+)III undergoes a steric repulsion involving its 7-hydroxyl group with an upper base if it approaches closer than 3.1 Å to the amino group of guanine.

DISCUSSION

Intercalation complexes having isomers of I oriented toward the amino group of guanine close to the model transition-state geometry should be particularly reactive. From Table I it is seen that the (+) isomers of both II and III have their epoxide groups oriented toward the 2-amino group of guanine, similar to the model transition-state geometry for both dinucleoside dimers. Moreover, these two intercalated stereoisomers also have the minimum distances between the atoms involved in the alkylation process: C(10) of I (defined as atom number 5 in Fig. 3) and the nitrogen of the amino group of guanine. The distances are 3.7 and 3.1 Å for (+)II and (+)III, respectively. Thus, the (+) enantiomers should be more reactive toward DNA if intercalation is a prereactive step in the alkylation process. This conclusion is consistent with experimental observation (3).

The intercalation complex involving (+)II should be expected from these calculations to be the most reactive state if intercalation is part of the reaction process. Table II summarizes the relationship between the mode of intercalation and location of (+)II relative to the base alkylation sites for IV. Clearly, mode d–e is critical to the explanation of the experimental observations (3). It should be noted also that the second most stable intercalation complex corresponds to the epoxide of II being close to the amino group of cytosine. The exocyclic amino group of cytosine is postulated to be the alkylation site for the minor cytosine–II adducts that are observed (9–12). These intercalation calculations may provide both a geometric and energetic base for this assumed reaction site. Stereo-stick models of the (+)II–IV complex are shown in Fig. 7. The transition-state orientation of the epoxide group relative to the 2-amino group of guanine is easily seen.

Table II—Intercalation Modes of (+)II with IV

Mode[a]	Intercalation Energy, kcal/mole	Position of Epoxide to Base
a–b	−84.00	cytosine-NH_2
c	−82.96	cytosine ring
d–e	−88.27	guanine-NH_2
f	−81.33	guanine-N(3)

[a] As defined in Fig. 5. The designation *i–j* indicates a transition-state geometry between two of the designated complexes.

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