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THE DOCKING MANOEUVRE AT A DRUG RECEPTOR: A QUANTUM MECHANICAL STUDY OF INTERCALATIVE ATTACK OF ETHIDIUM AND ITS CARBOXYLATED DERIVATIVE ON A DNA FRAGMENT

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Various semi-empirical quantum mechanical methods have been used to investigate the docking manoeuvre of ethidium and of its carboxylated derivative at the (dC-dG)·(dC-dG) receptor. The objective of the work was to determine whether the drug attacks the receptor in a random orientation or is pre-aligned for effective docking. An analogy was made between the interaction and two docking space vehicles. Charge distributions were computed for the intercalative site and the drug molecules; from these distributions it was possible to map, in three-dimensional space, the molecular electrostatic potential surrounding the receptor. Perturbation of the receptor fields by an approaching drug molecule showed field neutralization and a shifting local minimum as docking proceeds. Most of the electrostatic potential surrounding the receptor was shown to be derived from the two ionized phosphate groups. The orientation of the drug molecule was studied in a simplified anionic field constructed to reproduce that of the receptor phosphates. Rotation of ethidium and *p*-carboxyphenylethidium round the Eulerian axes in this simulated anionic field showed up distinct preferences for orientation of drug molecules in the vicinity of the receptor. Probability distributions for rotational populations demonstrated clearly that the receptor induces an orientation in the approaching ligand. The energy involved in modification of the alignment could be attributed to electrostatic interactions over large separation distances and to induced electron delocalization as the drug approaches closer to the receptor. This partition of the energy was considered further by monitoring electron migration in the drug molecules and analysis of dipole moment fluctuations. Orientation restrictions reflect entropy changes in the association reaction; these are discussed with respect to their importance in determination of reaction kinetics, and in two established models for drug–receptor interaction, namely, the 'lock and key' and 'zipper' mechanisms.

Introduction

The pharmacological analysis of drug–receptor interaction can be split into three problems. (*a*) How is the drug recognized and accepted by the receptor? (*b*) How does the drug–receptor complex form? (*c*) How does the complex exert its pharmacological action? This paper examines the first of these problems, that of drug attack at the receptor site. Specific interactions of drugs with receptors can be visualized, at their extremes, by two models (Burgen *et al.* 1975): first, the Fischer 'lock and key' model, in which the drug has an essentially rigid conformation and the spatial disposition of the atoms determines the potency and second, the 'zipper mechanism' in which the drug molecule has a fairly flexible chain structure. In the second model, conformational unfolding, in a series of steps, to fit the receptor site, is then of paramount importance. The extent to which a drug may fit a receptor has been investigated largely by hard sphere molecular models of the Corey, Pauling and Koltun type, which allow examination only of the van der Waals component of a final matched fit. For a reaction to

proceed efficiently, the mechanism of information transfer must satisfy the two criteria of speed and of the presentation of an optimal stereochemical configuration. The crucial step in the interaction of a drug with its stereospecific receptor would appear, therefore, to be recognition of a part of the desired conformation precisely aligned for binding. We wish to consider, in this study, whether the correct drug orientation is arrived at solely by chance, through a random collision process, or if the receptor is able to induce the ligand to adopt a suitable orientation *before* the two molecules come into van der Waals contact. If receptor-induced ligand orientation effects do occur, then it is reasonable to assume that they are mediated through electrostatic and quantum mechanical forces that operate on the close approach of two molecules.

The established concepts of chemical kinetics, such as the collision and transition–state theories, provide no detailed information about the interaction of associating molecules along their reaction pathway. Similarly, although experimental methods of molecular pharmacology have produced a wealth of information about numerous properties of drugs and of some of their associated receptors, there is a fundamental limit to experimentation. This limit stems from the quantum character of atomic structure and occurs when we desire further knowledge about the electronic and intramolecular properties of the molecules concerned. We can traverse beyond that limit, only by using those methods of theoretical chemistry that seek to determine and interpret the molecular wave functions. During the last decade, numerical quantum mechanics has developed sufficiently to make studies of molecular interactions possible. We have used some of these methods to explore the quantum mechanical principles that govern the orientation of a drug molecule before complex formation with the receptor.

In the choice of a suitable drug–receptor system to examine, two facts must be borne in mind; the molecular structure of the receptor must be known and the active unit of the receptor must be small enough to make quantum mechanical computations feasible. Thus, as a starting point, the X-ray crystal structures of the drug molecule and the receptor are needed. These requirements narrow the field of choice considerably and we have taken the intercalative attack of the trypanocidal phenanthridinium drug, ethidium, on DNA as an illustrative study of the principles governing the docking of a drug at its receptor. The term 'docking manoeuvre' is used here to describe the receptor-induced ligand orientational effects, by analogy with the interaction between two approaching space vehicles, for which there is both a convenient terminology and a set of principles by which the manoeuvre can be understood.

The field of drug–nucleic acid interaction is largely dominated by the intercalation mechanism of binding, wherein planar aromatic portions of drug molecules are sandwiched between adjacent base pairs of the polynucleotide helix. This mode of interaction has been postulated for many drugs and antibiotics. Substances of quite diverse structure have been shown to be capable of intercalation: compounds such as acridine and phenanthridine drugs, anthracycline, quinoxaline and phenoxazone antibiotics, and carcinogenic polycyclic aromatic hydrocarbons. Drugs that bind to DNA in this way affect its structure and function in a manner that prevents its participation as a template in nucleic acid synthesis. These drug compounds have found great use in veterinary and clinical practice for the treatment of protozoal, parasitic and neoplastic diseases. However, there are no obvious answers available to explain this remarkable range of selective toxicity. Consequently, interest has centred on the physical chemistry of the interaction of these drugs with their putative biological receptor, to try to discover the origin of the selective chemotherapeutic effect.

There are many advantages in studying this particular drug–receptor system. DNA is a

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vital target for drug action, wherever cell reproduction is to be stopped, since transcription of the genetic code from DNA is prevented at an intercalated site. DNA may be viewed, pharmacologically, as a polymer of intercalative drug receptor sites which vary only by the sequential arrangement of the pyrimidine and purine bases. Hence, despite the fact that the receptor is, in actuality, a macromolecule, the active site (to which the drug will bind) may be considered as a simple dinucleotide in which the phosphodiester backbone is extended and the base pairs unwound and unstacked, thereby creating a cavity between them, into which the drug molecule intercalates. These conformational changes are induced in the polymer by binding of ethidium; the helix unwinds by 26° for each drug molecule intercalated (Wang 1974). X-ray fibre structures of DNA and of RNA are known, as, indeed, are the crystal structures of deoxyribose derivatives of the bases. The simplest receptor unit is the double helical dinucleotide, for which there are crystal structures of (G–C)·(G–C), ((iodo–C)–G)·((iodo–C)–G)–ethidium, ((iodo–U)–A)·((iodo–U)–A)–ethidium complexes. Furthermore, extensive studies of the kinetic, thermodynamic and structural properties of ethidium binding to DNA have been performed. Therefore, there is the possibility that results from quantum mechanical computations may be related to known experimental data, and any predictions derived from the work may be tested.

Molecular quantum mechanics provides useful information about conformational energies and probabilities, the electron distribution in a molecule, the molecular electrostatic potential resulting from the electron distribution, bond and total dipole moments, energy levels of occupied and virtual orbitals and molecular interaction energies. All these theoretically determined parameters are used in this paper to probe into those factors that determine the orientation of a drug molecule in the field of its receptor. The paper is written in nine sections, which to assist the reader, are outlined briefly here. First, a geometry for an intercalative site is constructed by using the self-complementary base paired dC-dG dinucleoside monophosphate (§1). From this geometry, the overlapping-fragment technique employing CNDO/2 calculations is used to derive the electron distribution of the receptor (§ 2). Charge distribution calculations are made for two intercalative drug molecules, ethidium and *p*-carboxyphenylethidium (§ 3). These two drugs have been chosen because ethidium is positively charged and *p*-carboxyphenylethidium is electrically neutral and a zwitterion; the intercalative properties of both are well known. From the charge distribution of the receptor, the electrostatic fields are generated in the surrounding space (§ 4). The mutual interaction of the molecular electrostatic potentials for the receptor and for ethidium is examined as the drug approaches the receptor (§ 5). In § 6, a decision, based upon electrostatic considerations, is made concerning the best side to attack the intercalative site. With this information in hand, docking manoeuvre rotations around the three coordinate axes are performed, at distances of 6 and 12 Å†, for ethidium and for *p*-carboxyphenylethidium in a simulated anionic field (§ 7). Subsequent analysis shows that the interaction energy differences in docking alignment can be partitioned into discrete energy contributions, with major components from electrostatic and delocalization energies (§ 8). Furthermore, subtle changes in molecular orbital energies for translation of ethidium to the receptor are observed in § 9. These findings are discussed in terms of their general relevance to the docking manoeuvre as a pharmacological problem, and with their application to the phenomenon of intercalation.

† $1\ \text{Å} = 10^{-10}\ \text{m} = 10^{-1}\ \text{nm}$.

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Computational methods

To investigate the docking manoeuvre at a drug receptor, two standard semi-empirical molecular orbital methods have been used, complete neglect of differential overlap, CNDO/2 (Dobosh 1969), and perturbative configuration interaction using localized orbitals, PCILO (Claverie *et al.* 1972). CNDO/2 is a linear combination of atomic orbitals procedure employing all the valence electrons, inner shells being treated as part of a rigid core, so that they modify the nuclear potential in the one electron part of the Hamiltonian. This method has been adequately documented by Pople & Beveridge (1970). This program has been used to calculate the electron distribution of molecular fragments, to create a charge description of the receptor (dC-dG)·(dC-dG). The PCILO method is a variation of the semi-empirical calculation employing configuration interaction and perturbation of the zero-order wavefunction. The use of localized molecular orbitals introduces information about structure, contained in the chemical formula at the start of the calculation. This notion is based on the observation that the interaction between localized molecular orbitals decreases rapidly as the distance between them increases. A full description of PCILO philosophy is given by Malrieu (1977).

Molecular structure is entered into the molecular orbital programs either as Cartesian coordinates of the atoms, or in terms of standard geometry. An axis transformation program, ORTHOG, was written to convert fractional crystal coordinates to Cartesian coordinates. This routine linked to the ATCOOR program of Nordlander (1974) was used to express the structure in geometrical terms.

Molecular electrostatic potentials have been used to illustrate the electrostatic fields surrounding the drug receptor and the interacting drug molecule. These potentials were constructed by interaction with a proton at points on a grid overlaid on the molecular structure by the approximate method vss of Giessner-Prettre (1974). The charge distribution and atomic coordinates are used as input. Details of the method are given in the paper by Giessner-Prettre & Pullman (1972). Display of the output from this program is a very difficult problem for a complex structure, since one is trying to portray a fluctuating field intensity in the three spatial dimensions. We have adopted three graphical procedures in an attempt to analyse the electrostatic fields. (*a*) Fields were contoured through a single plane of interest; the contour method involved interpolation along the diagonals and edges of each grid cell. (*b*) Relative field intensity in a single plane has been expressed as a hidden line supression plot by the travelling boundary method. (*c*) Three-dimensional surface shapes for a single energy value have been drawn by contouring through successive planes of a block of space surrounding the molecule. These contours were then displaced and the block skewed to give an impression of depth to the potential surface.

Interaction energy plots for the docking manoeuvre (§ 7) were smoothed between the calculated values by a cubic-spline fit over three points, with the curvature held constant at each point. All computation was carried out on the University of Cambridge IBM 370/165 computer.

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Results and discussion

1. *Construction of the geometry of the dinucleotide receptor fragment* (dC-dG)·(dC-dG)

Studies of binding of ethidium to dinucleotides have shown that the drug has a clear preference for binding to pyrimidine–purine sequence isomers and that it is C-G that binds ethidium the most strongly (Krugh *et al.* 1975; Krugh & Reinhardt 1975). The self-complementary dC-dG mini-helix was therefore chosen as a suitable receptor for a study of some of the properties of the docking manoeuvre. The mechanism of intercalation of ethidium into DNA involves binding of the drug to the polymer directly from the free solution state, without conversion from an externally bound intermediate. Considerations of the internal flexible motions of DNA suggest that the intercalation cavity may occur spontaneously and that

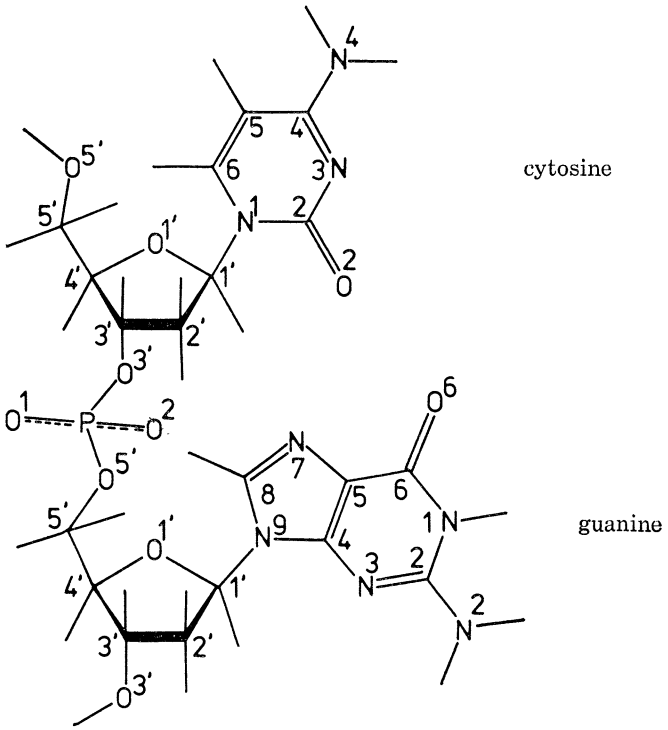

Figure 1. Atom labelling for dC-dG half of the self-complementary mini-helix.

ethidium inserts into a preformed binding site (Li & Crothers 1969; Bresloff & Crothers 1975; Sobell *et al.* 1978). As yet, there are no X-ray crystal structure determinations for deoxydinucleoside monophosphates in either the Watson–Crick duplex or in the unwound and unstacked helical state when bound with intercalating drugs. However, several crystal structures are known for double helical RNA dinucleotides and for related dinucleotide complexes with ethidium (Rosenberg *et al.* 1976; Seeman *et al.* 1976; Jain *et al.* 1977; Tsai *et al.* 1977). It is known that quantum mechanical studies are critically dependent on molecular geometry and that input from standard bond lengths and bond angles may give results quite different from input information derived from exact crystal geometry. Various attempts have been made previously to build intercalation binding-site geometries (Sobell *et al.* 1977; Berman *et al.* 1978; Alden & Arnott 1975, 1977; Bond *et al.* 1975); these were not used, for the reasons that RNA dinucleo-

tides are not in the B-DNA conformation and that the theoretically constructed intercalation sites do not have the experimentally determined unwinding angle of 26° for ethidium (Wang 1974). Therefore, the procedure adopted has been to construct a deoxydinucleotide, unwound and with unstacked base pairs, from smaller fragments of known crystal structure. The pieces were assembled to form the double helical receptor in which only dihedral angles were changed. Crystal geometries for the bases and for the deoxyribose sugars were taken from the nucleotide determinations for deoxycitidine (Viswamitra *et al.* 1971) and for deoxyguanosine 5′-monophosphate (Young *et al.* 1974); bond lengths and bond angles for the phosphate group were taken from the self-complementary mini-helical dinucleotide G–C (Rosenberg *et al.* 1976). The cytosine and guanine bases were arranged in a Watson–Crick base pairing scheme. The bases were made planar and normal to the helix axis positioned as in B-type DNA (Arnott *et al.* 1969), rotated around the helix axis so that the winding angle was 10° (equal to the experimentally determined value for ethidium binding to polymeric DNA) and then separated, by 6.8 Å, along the helix axis. Twist and tilt in the base planes was neglected, to simplify the geometry calculations. An acceptable conformation for the sugar phosphate backbone was computed in the following way (figure 1). Both deoxyribose rings were rotated round their glycosidic bonds and the O5′G atom was rotated round the C4′G–C5′G bond. When the interatomic distance between O3′C and O5′G was equal to or less than that interatomic distance for the same atoms in the G-C crystal structure (Rosenberg *et al.* 1976), then the phosphorus atom was rotated round O3′C–C3′C until bond angles O3′C–P–O5′G and P–O5′G–C5′G and bond length P–O5′G occurred equal to those found for G-C (Rosenberg *et al.* 1976). A further criterion of acceptability for the geometry of the intercalative site was that the non-ester-linked phosphate oxygen atoms must point in a direction away from the helix axis.

No steps were taken to form a repeating unit from the dinucleotide. It is acknowledged that many different structures could be constructed by this method; no attempt has been made to distinguish between these structures on grounds of minimum energy considerations. Views of the skeletal geometry of the dinucleotide can be seen in figures 4, 5 and 6. It must be remembered that, throughout the rest of this paper, the concepts illustrated in the docking manoeuvre are related to this geometry only and are representative of principles, rather than of calculations for the actual natural structure.

2. *Charge distribution of the* (dC-dG)·(dC-dG) *receptor*

The residual charge distribution for a large molecule like (dC-dG)·(dC-dG) cannot be obtained from a single molecular orbital calculation; therefore, the principle of overlapping fragments has been used to derive the total electronic population. CNDO/2 calculations using the geometry previously described were performed on the following moieties: cytosine, guanine, a cytosine·guanine base pair, cytosine deoxyribose, guanine deoxyribose and deoxyribose–phosphate–deoxyribose. A comparison was made between the net charge distribution of each segment taken in isolation and that determined with a larger fragment. Maximum charge differences were detected in the areas of overlap; for example, in the hydrogen-bonding region of the base pairs, the distribution varied by 0.04*e* compared with the atomic charges in the separate pieces. However, the difference in charge diminished rapidly on moving a short distance from the adjoining atoms, being less than 0.005*e* two to three bonds away. Therefore, it is possible to construct the net atomic charge distribution for (dC-dG)·(dC-dG) from the

fragments, with simultaneous substitution of the charges for those atoms in the overlap region (figure 2). A further partition of charge into σ- and π-components can be obtained from the density matrix of CNDO/2 calculations; these are tabulated, for the phosphate group, in table 1.

An interesting feature of the C·G base pair is the alternating sign distribution on all adjacent atoms except N7 and C5 of guanine, which are both negative and could not possibly be alternating in a five-membered ring. The heavy atoms are considerably polarized and have charges

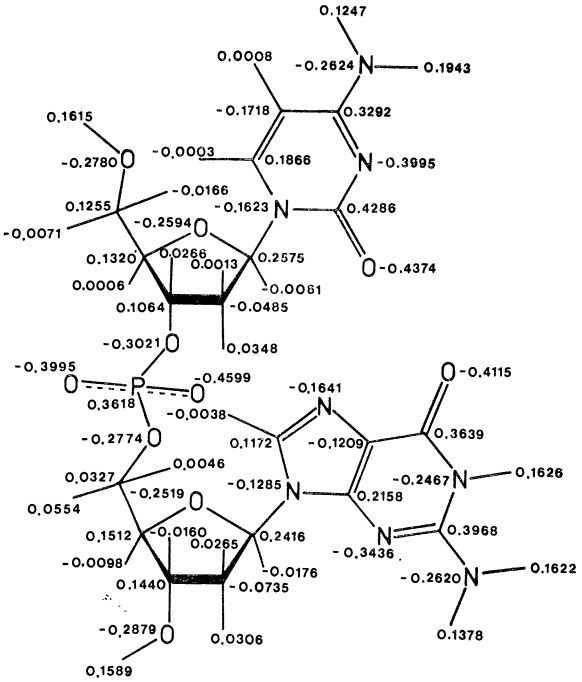

FIGURE 2. Net atomic charge distribution for dC-dG.

TABLE 1. ORBITAL POPULATIONS IN THE PHOSPHATE GROUP

(See figure 1 for identification of atom labels. The total p-orbital population in phosphorus is 1.7935 electrons and the total d-orbital population is 1.8604.)

	orbital								
atom	s	p_x	p_y	p_z	d_{z^2}	d_{xz}	d_{yz}	$d_{x^2-y^2}$	d_{xy}
P	0.9843	0.5769	0.6304	0.5862	0.3379	0.3841	0.3979	0.3982	0.3423
O3′C	1.6195	1.6541	1.6728	1.3557					
O1 P	1.7647	1.6035	1.5364	1.5553					
O2 P	1.7508	1.5477	1.4925	1.6086					
O5′G	1.6899	1.5153	1.2909	1.7813					

greater than $\pm 0.12e$. Hydrogen atoms attached to nitrogen are more polarized than those linked to carbon; this reflects the relative electronegativities of the heavy atoms. The amino nitrogen atoms N4C and N2G have their lone–pairs orthogonal to the base plane and may be involved in electron donation to a positively charged chromophore intercalated between the bases. These results for the calculated charge distribution of the C·G base pair region are in qualitiative agreement with those obtained by CNDO/2 procedures for isolated base pairs (Pullman & Pullman 1969; Pack & Loew 1978). Charge computations have not been carried

out previously for the sugar–phosphate backbone in the open configuration. The deoxyribose rings have very similar charges, the only major differences occur between the C3′ and C5′ positions, where the atoms attached are not the same. The furanose oxygen has a net charge of about $-0.25e$ and could possibly make hydrogen bonds to an intercalated group such as NH_2 of ethidium. The single charge of the anionic phosphate oxygen is delocalized throughout the group, which has a net charge of $-1.08e$. The excess charge is held entirely in the p-orbitals of the four oxygen atoms (table 1). The positive charge on phosphorus results from a loss of electrons from the p- and d-orbitals. It is worth noting that the charge on the carbonyl oxygen atoms of the base pairs is similar to that on the non-ester-linked phosphate oxygens and emphasizes the marked polarity of the carbonyl bond. The carbon atoms of deoxyribose are only slightly charged and the most positive is that in the glycosidic bond.

3. *Charge distribution of ethidium and p-carboxyphenylethidium*

The ground state electronic properties of ethidium were calculated using the PCILO method together with the crystal geometry of ethidium bromide (Subramanian *et al.* 1971). In this structure, the phenanthridinium ring is slightly non-planar; the phenyl ring is set to 97° with respect to the mean plane, and the terminal carbon atom of the ethyl substituent is 84° out of

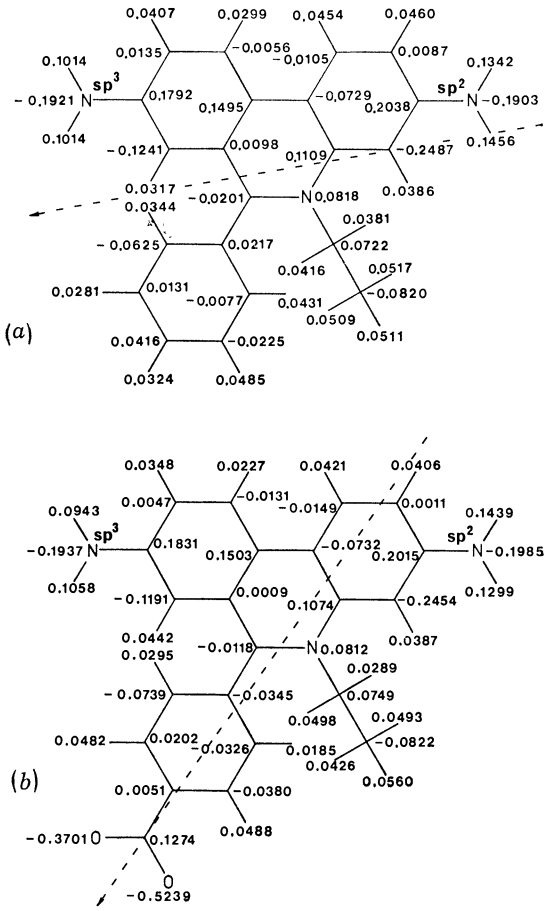

FIGURE 3. Net atomic charge distribution for ethidium and *p*-carboxyphenylethidium: (*a*) ethidium; (*b*) *p*-carboxyphenylethidium. The direction of the dipole is indicated.

the plane. The amino nitrogen atom at the 8-position is sp^3-hybridized and conformational analysis shows that the hydrogen atoms lie on the opposite side of the phenanthridine plane to the ethyl group and that the nitrogen lone–pair is at right angles to this plane (Wakelin & Dean 1979*a*).

Figure 3 illustrates the electron distribution in ethidium, obtained from the final density matrix, with allowance for full optimization of bond polarities. The main characteristic of the distribution is the extensive delocalization of the positive charge, which is classically thought to reside on the quaternary nitrogen; instead, it has a charge of only $+0.0818e$. Proportionation of the net residual charge distribution between the functional groups of the molecule reveals that the ethyl group carries a charge of $+0.2236e$, the phenyl ring one of $+0.1702e$, the amino groups, $+0.1002e$, and the phenanthridinium ring itself, $+0.5067e$. A similar analysis for the non-quaternized molecule shows that the net charges on these groups are essentially zero. Thus, in ethidium, the majority of the positive charge is extensively delocalized over the amino-phenanthridine portion of the molecule. However, as much as 40% spreads to the exocyclic phenyl and ethyl groups. Further comparison of the average net charge per hydrogen atom in ethidium ($+0.0567e$) with that in the non-quaternized molecule ($+0.0450e$) indicates that as much as 26% has been delocalized onto these atoms. Furthermore, the amino hydrogens are quite positively charged with approximately 0.10–0.15e charges per atom. This pattern of the residual charges, outlined above for ethidium, is common to many phenanthridines, phenazines and acridines (Wakelin & Dean 1979*a*, *b*), irrespective of whether the ring nitrogen is protonated or alkylated.

As a result of the considerable charge separation in ethidium, together with non-negligible contributions arising from atomic orbital hybridization, the molecule has a large dipole moment (24.59 D). In figure 3, the dipole moment is seen to lie within 20° of the major axis of the chromophore and is inclined at 5° with respect to the plane of the phenanthridine ring.

To investigate the electrostatic field effects of the drug upon the docking manoeuvre, calculations were performed with the *p*-carboxyphenyl derivative of ethidium. The electron distribution of this compound (figure 3) was calculated using the PCILO method, with the same initial geometry as ethidium to which had been added the carboxylate group, with use of standard bond lengths and bond angles. This charge distribution may be compared with that of ethidium; again the positive quaternary charge is extensively delocalized over many atoms, whereas the negative charge of the zwitterion lies almost entirely on the carboxyl group itself. The net distributions on the functional groups may be summarized as follows: the ethyl group retains $+0.2193e$ charge, the charge on the phenyl ring becomes $-0.0087e$, the amino groups, $+0.0817e$, and the phenanthridinium ring, $+0.4758e$; the carboxyl group carries a charge of $-0.7666e$. The average residual hydrogen charge remains very similar to that of ethidium ($+0.0567e$), being $+0.0562e$. Comparison of the net group charges reveals that, in the zwitterion, the effects of the ionized carboxyl group are largely confined to the carboxyphenyl moiety, with only 0.0537e redistributing into the remainder of the molecule. The dipole moment is considerably larger than that of ethidium, being 33.98 D. The direction of the moment has rotated, somewhat, compared to that of the parent molecule and now lies closer to the minor axis of the chromophore and is within approximately 2° of the plane of the phenanthridine ring.

Thus, in summary, although there are slight inequalities in charge distribution in the amino-phenanthridine parts, the major differences lie in the gross separation of negative and positive

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charges. Hence, these compounds are excellent candidates for a comparitive study of orientation effects in the docking manoeuvre that result from a local charge variation in the attacking drug molecules.

4. *The molecular electrostatic potential emanating from the* (dC-dG)·(dC-dG) *receptor*

Previous receptor mapping procedures have been based on correlation of charge distributions with potency in a series of drug molecules and then inference of a complementary electrostatic field in the receptor. The results of this approach, although of practical use to pharmacologists, are questionable since the receptor structure remains unknown. When a plausible tertiary configuration of the receptor binding site is available we are in a position to chart the radiating fields correctly on sound quantum mechanical grounds. In this section, the molecular electrostatic potential surrounding the (dC-dG)·(dC-dG) receptor in an open conformation ready to receive an intercalative drug, is computed by the vss method.

The (dC-dG)·(dC-dG) was placed centrally in a cubic block of space, side length 20 Å, and the interaction of a unit positive charge with the electron distribution of the receptor was calculated at regular three-dimensional grid points in this volume. In all, 158 000 grid positions were used. The resultant electrostatic field has four dimensions, field magnitude and three spatial coordinates, and is thus impossible to illustrate on a two-dimensional surface. For a simple molecule, a limited representation of a fourth dimension can be achieved by using different colours to represent field strength and then drawing their shapes with perspective (Dean 1979). However, for a very complex structure like (dC-dG)·(dC-dG), visual intelligibility limits the graphical representation to pictures of a single potential. These surfaces are, in fact, the receptor fields 'seen' by an approaching drug molecule. A detailed study of them should reveal the essential features of a long range electrostatic guidance mechanism operating between the receptor and attacking drug molecules.

Two contour values have been chosen to illustrate the three-dimensional shapes of these electrostatic fields. The -334 kJ mol^{-1} contour is a continuous surface which surrounds both phosphate groups, and sweeps in between the base planes (figure 4*a*, *b*, plate 1), whereas the -418 kJ mol^{-1} contour is localized to the region of the sugar–phosphate backbone on either side of the intercalative site (figure 4*c*, *d*, plate 2). Both contours are displayed from the wide and narrow groove sides. Figure 5*a*, plate 3, complements these illustrations since it is a contour section through the mid-plane between the unstacked bases and is the plane of intercalation. From figures 4*a*–*d* it can be seen that the potential surface is dominated by the ionized phosphate groups, near to which lies the global minimum of -761 kJ mol^{-1}; for this geometrical structure, these fields point predominantly into the narrow groove. The fields in the wide groove terminate abruptly, close to the sugar–phosphate backbone. Local perturbations around the base planes can be observed, for example an isolated spherical shell in the region of O6G and O2C (figure 4*c*, *d*) indicates that there is a considerable electrostatic potential generated by the carbonyl oxygen atoms of the base pairs. Similarly, the N3G has an associated high potential. The non-hydrogen-bonded 4-amino hydrogen atom of cytosine is not surrounded by a large electrostatic field attractive towards a proton. These fields therefore mirror, to a large extent, the charge distribution of (dC-dG)·(dC-dG). The -334 kJ mol^{-1} contour indicates that a positively charged molecule would not only be attracted by electrostatic forces towards the intercalative site, but would also experience a favourable pull into the cavity between the base

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planes. Once there, it would then be held electrostatically in a sandwich state suitable for bonding by stacking interactions with the base pairs. This concept is dealt with at length in the next section.

5. *Perturbation of the receptor electrostatic field induced by ethidium*

In the previous section an attempt, somewhat incomplete because of the molecular complexity, was made to portray the three-dimensional shapes of the electrostatic fields surrounding the isolated receptor. More detail can be obtained by means of multiple contours through many planar sections of the receptor molecule, but this would be a cumbersome procedure. To clarify the problem, a few definitions are needed to establish the terminology of a docking manoeuvre at a drug receptor. Essentially, it is analogous to the well defined event between two space vehicles. Let the drug and its receptor have their structures described respectively by a right- and a left-hand Cartesian coordinate system. Line up one of the major axes, say the x-axis, so that the origins of the two objects are at $+x$ in the other local coordinate frame and so that the y-and z-axes in both systems are coincident in the fully docked position. Then rotation round the x-axis is termed roll, rotation round the y-axis is yaw and rotation round the z-axis is pitch (figure 8). The leading edge is nearer to, and the trailing edge is further from, the other object. The flight path is the direction down the translational axis (x) and the glide plane lies along this trajectory, with a yz coordinate of the final docked orientation. Thus, we can describe fully the relative motion of two interacting molecules.

The six illustrations of figure 5*a–f*, plates 3–5, are contour displays of the resultant electrostatic field in the intercalation plane, with the attacking drug molecule at fixed distances from its final resting position in the receptor. The frame size is 20 Å by 30 Å and computations were performed on 5551 grid positions, before graphical display with the contouring routine. Justification for the choice of the relative alignment of the molecules is given in a later section, where energy calculations are performed for roll, yaw and pitch angles. The flight path direction has been determined from probability calculations of proton interaction energies and, for this geometry, lies on the narrow groove side. The orientation of the fully intercalated ligand has been set to have the N=C bond of the phenanthridinium ring lying directly between the nitrogen atoms of the 2-amino groups of guanine; this is the position found in the crystal complex of ((iodo–C)–G)·((iodo–C)–G)–ethidium (Jain *et al.* 1977).

The grid frames contoured in figure 5 can be thought of as instantaneous events taken from a kinetic sequence of the drug travelling along the glide plane and docking into the receptor. Contours are given at $+418$ kJ mol^{-1} (red), 0 kJ mol^{-1} (green) and at intervals of -50 kJ. mol^{-1} (black); the narrow groove faces the lower part of the frame. At infinite separation of ethidium and (dC-dG)·(dC-dG), the electrostatic fields are very nearly symmetrical about the flight path (figure 5*a*). However, there are distinct differences between the grooves. The bulk of the electrostatic potential is found on the narrow groove side, near the phosphate groups, where the energy minimum is -761 kJ mol^{-1}. The -350 kJ contour spans the base pair region in the wide and narrow grooves. Along the flight path, a local minimum is found, at 4 Å from the helix axis, in the narrow groove; attention is drawn to this since it will be seen later that this minimum shifts its position as the drug molecule glides in. Regions of repulsion towards a proton are found where the contour plane intersects the bonds in the phosphate and deoxyribose CH_2OH groups. The data from this computation has been converted to a hidden line suppression profile, drawn with perspective on an arbitrary scale in figure 6*a*, plate 6,

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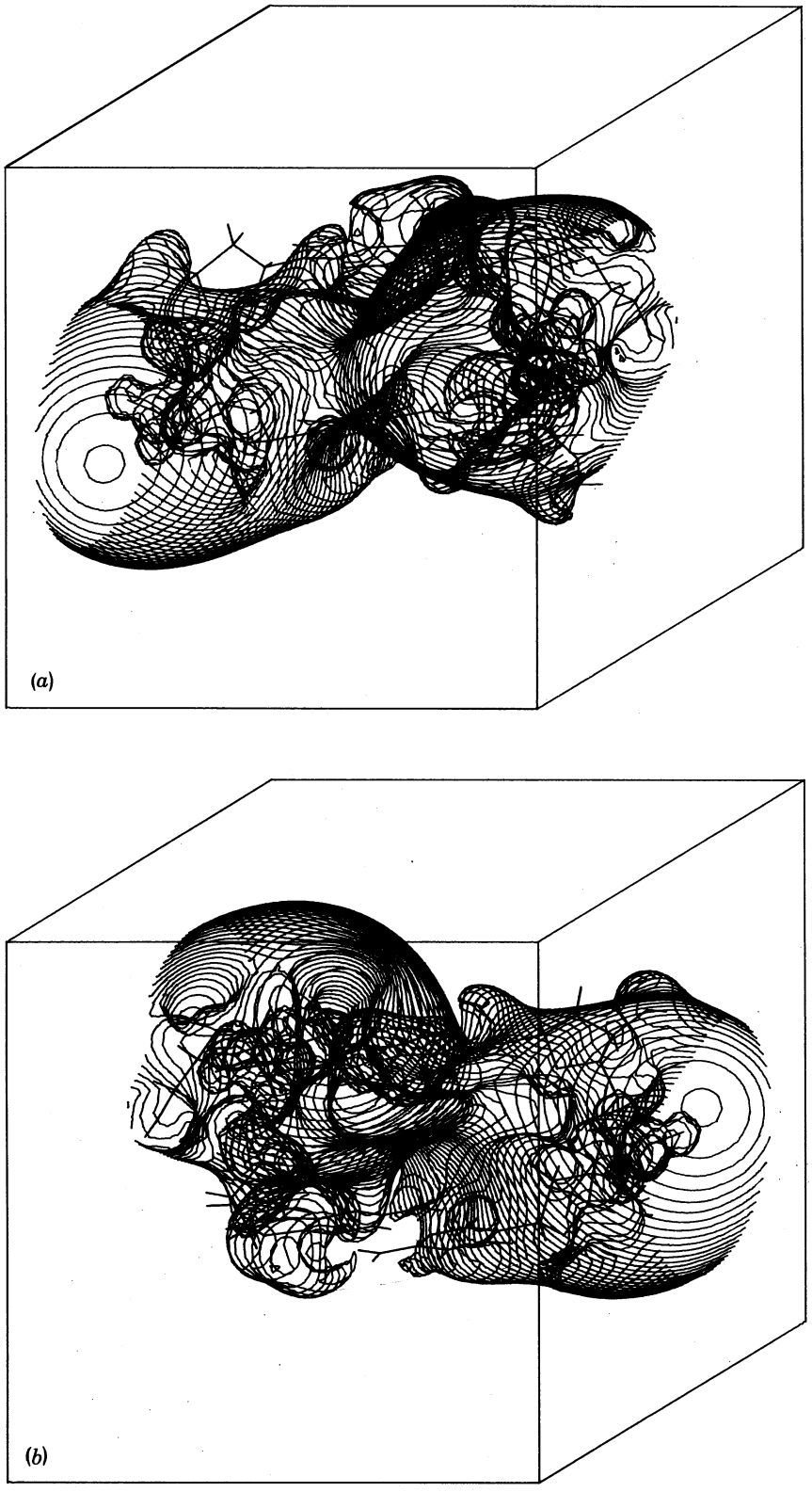

FIGURE 4. Three-dimensional molecular electrostatic potential surfaces for the receptor (dC–dG) · (dC–dG). Bonds are shown by the skeleton drawings superimposed on the field contours. Potential surface –334 kJ mol^{-1} with (*a*) the narrow groove and (*b*) the wide groove towards the front face.

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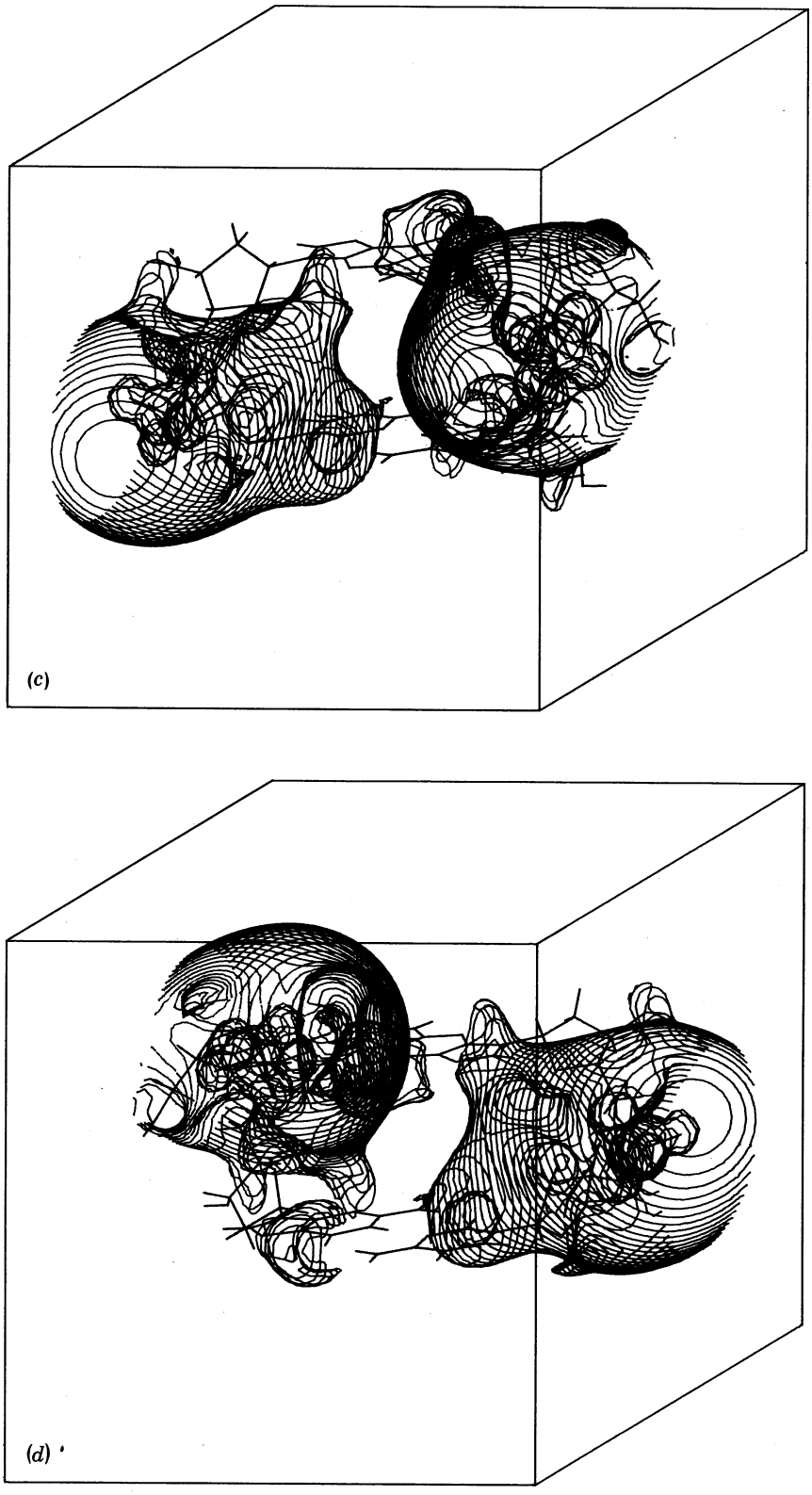

FIGURE 4 (continued). Potential surface –418 kJ mol^{-1} with (*c*) the narrow groove and (*d*) the wide groove towards the front face.

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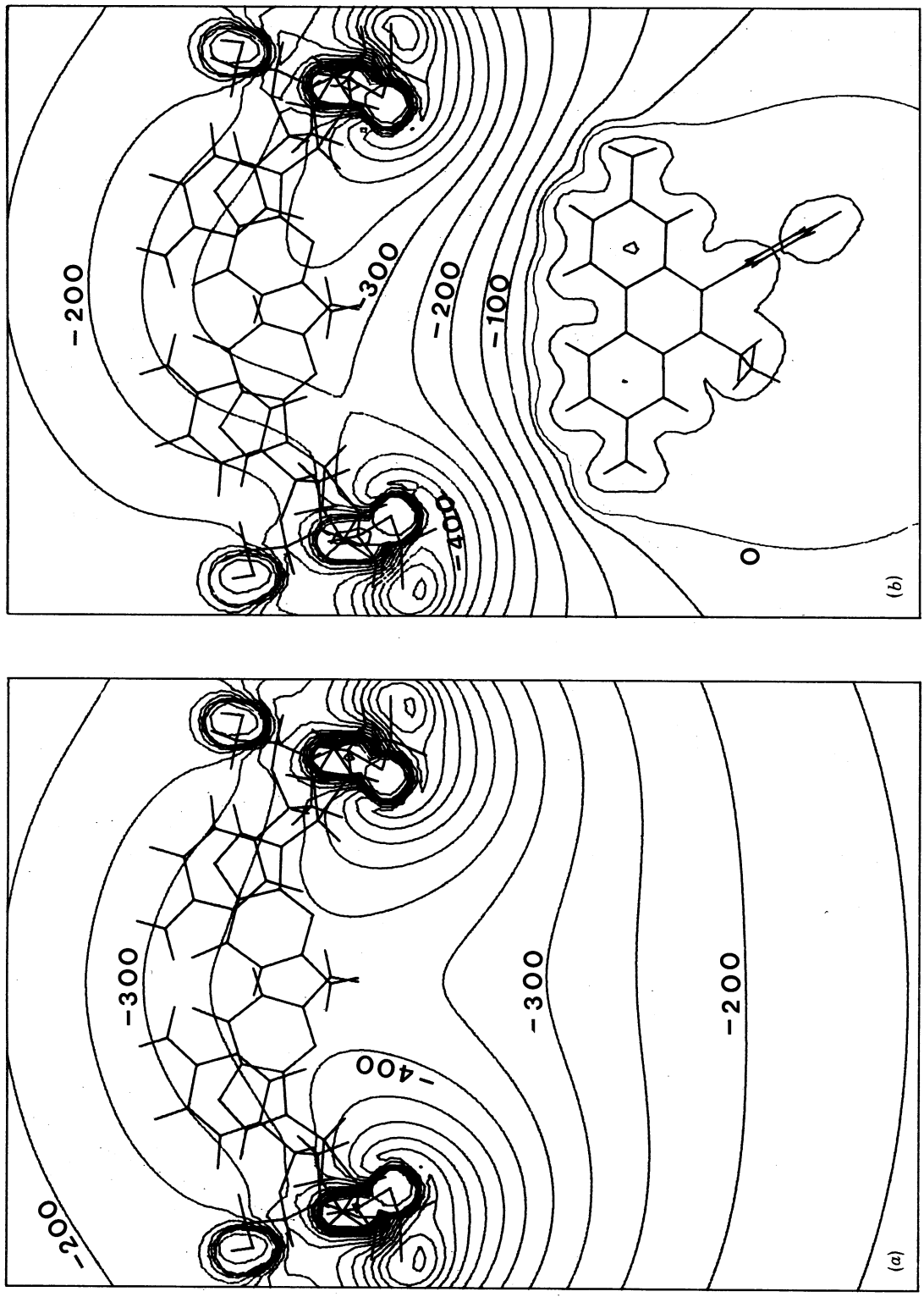

FIGURE 5. Electrostatic contour maps through the intercalation plane of the receptor. Narrow groove facing the lower part of the frame. Red contour +418 kJ mol^{-1}, green contour 0 kJ mol^{-1}, black contours at –50 kJ mol^{-1} intervals. (*a*) Isolated receptor. (*b*) Ethidium at 12Å from the intercalated position.

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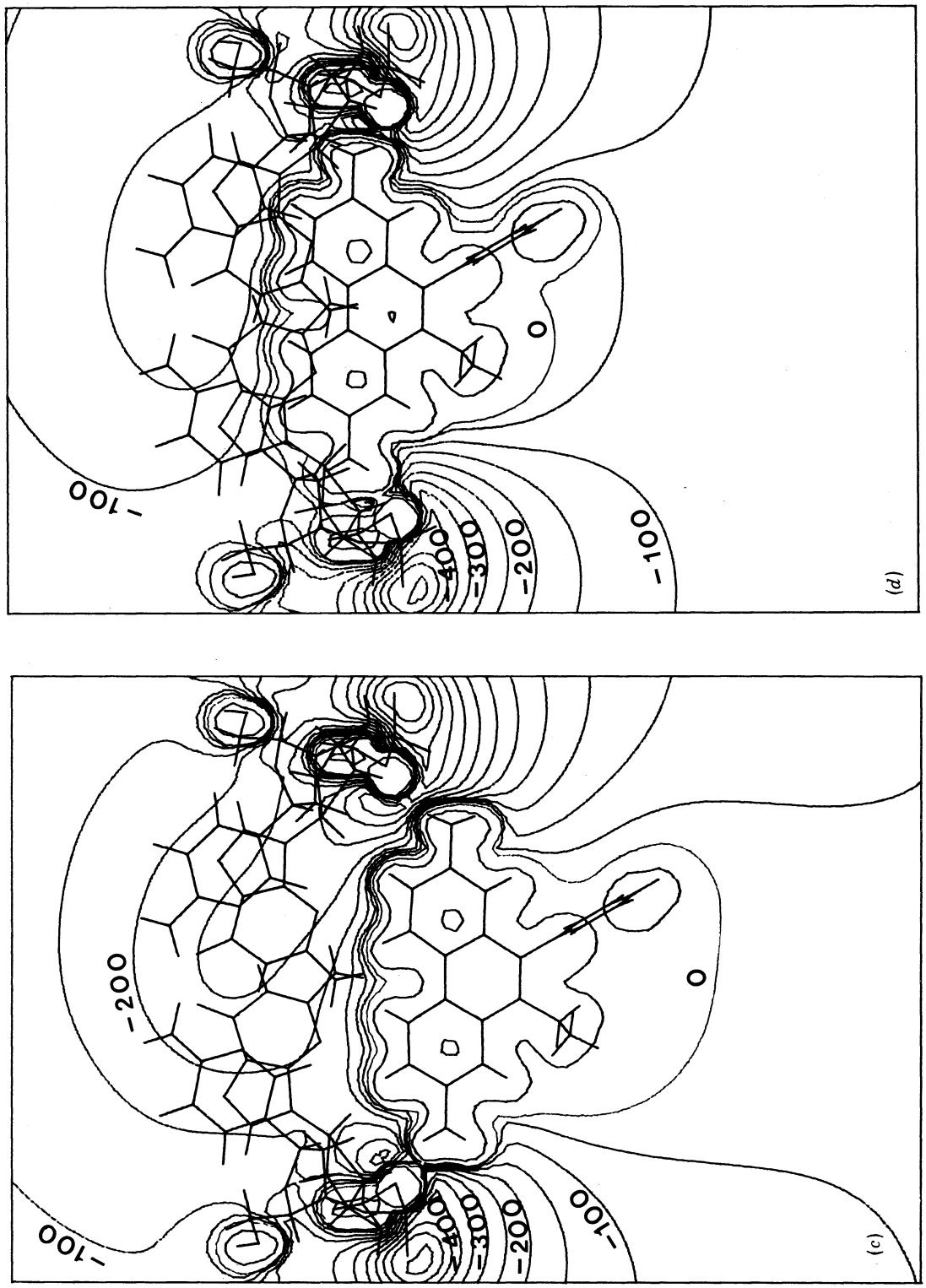

FIGURE 5 (continued). (*c*) Ethidium at 6Å from the intercalated position. (*d*) Ethidium at 3Å from the intercalated position.

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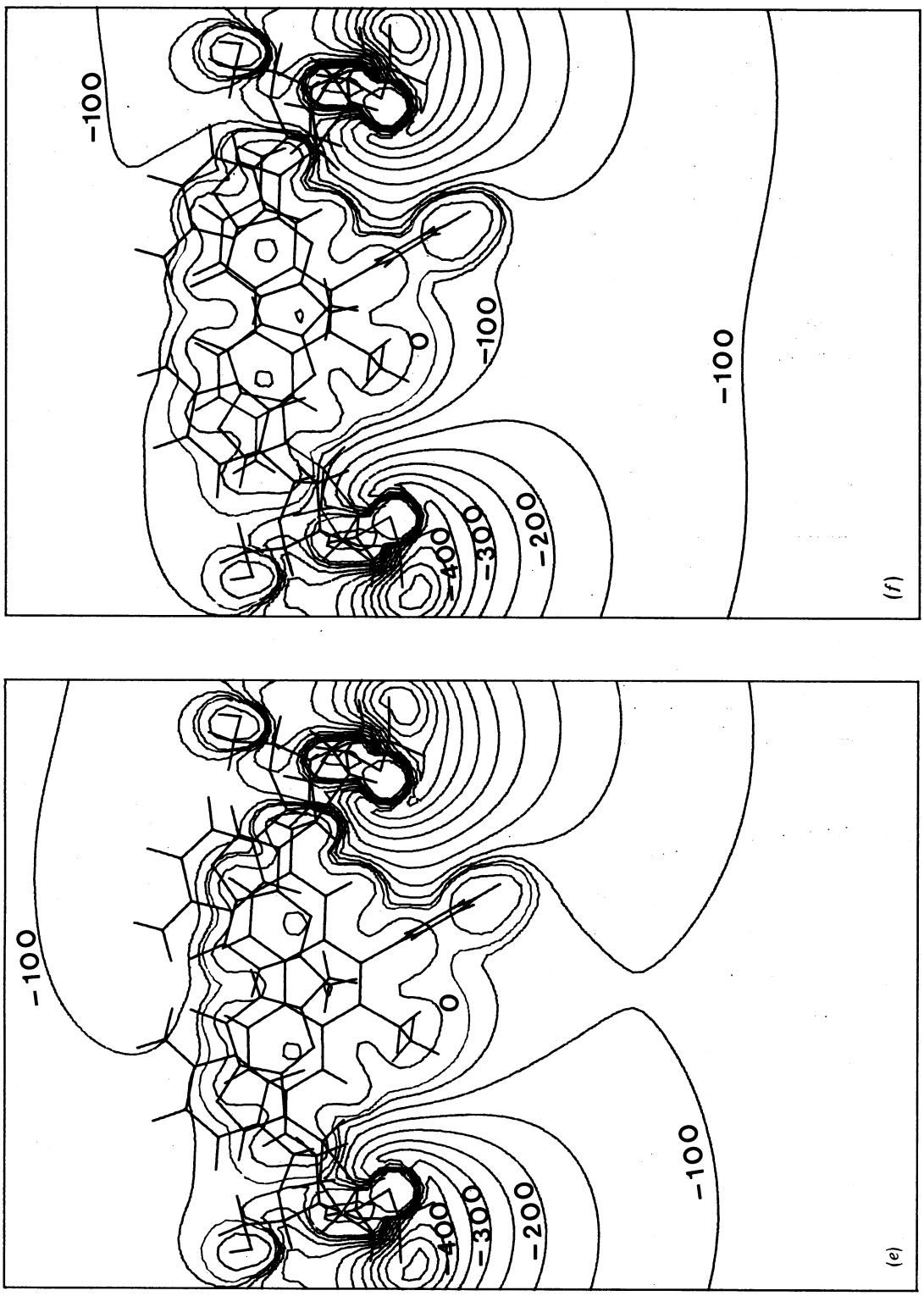

FIGURE 5 (continued). (*e*) Ethidium at 1Å from the intercalated position. (*f*) Ethidium fully intercalated.

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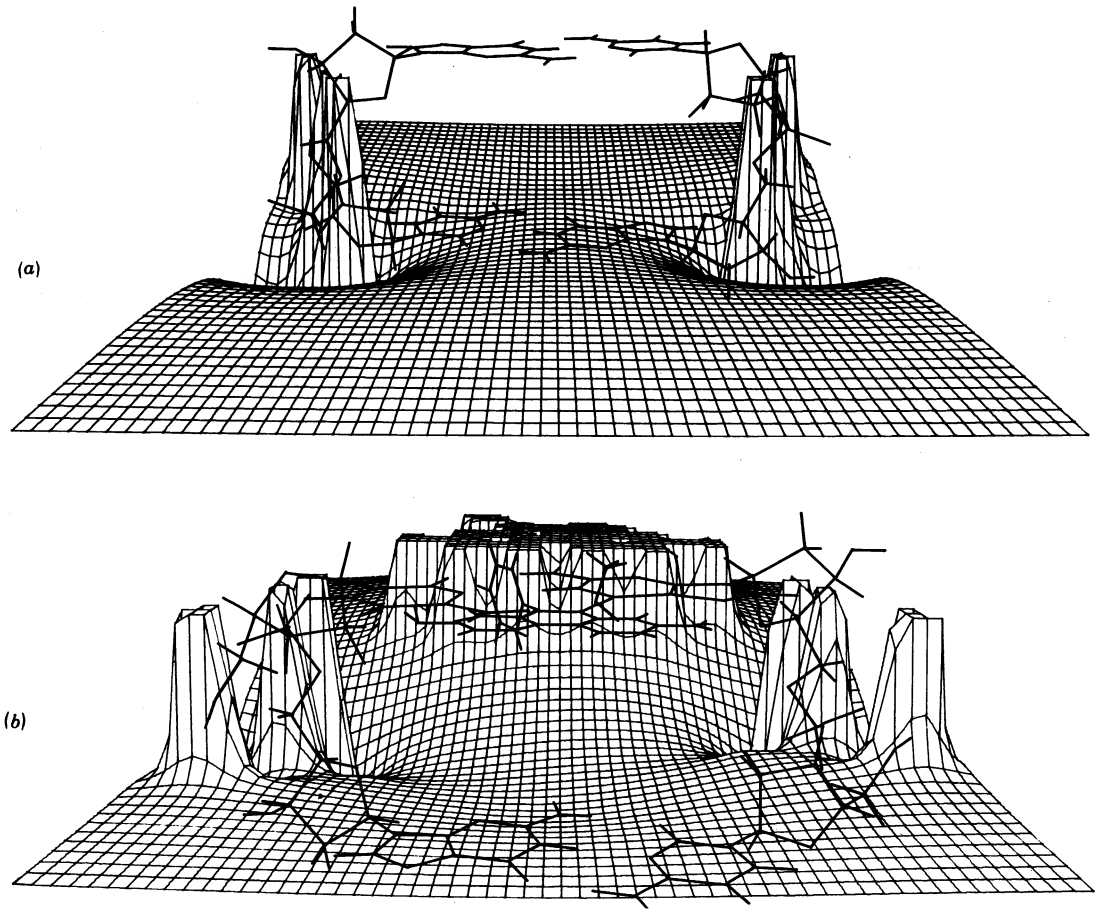

FIGURE 6. Hidden line suppression profiles of the electrostatic potential in the intercalation plane. (*a*) Data taken from figure 5*a* and viewed through the receptor from the narrow groove side. (*b*) Data taken from figure 5*b* and viewed through the receptor from the wide groove towards the docking ethidium molecule.

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where the observer is facing the helix axis from the side of the narrow groove; it demonstrates the relative magnitudes of the electrostatic field. A rapid descent into the potential wells is clearly seen near the phosphates. Peaks occurring where the graphing plane sections the chemical bonds are truncated at 418 kJ mol^{-1}. A shallow depression along the flight path between the bases can be discerned, which is the minimum energy position for an interacting proton. This plot is the 'drug's-eye view' of the receptor fields generated down the glide plane.

A comparison between figure 5*a* and *b* illustrates the effect on the electrostatic field of placing the positively charged ethidium molecule 12 Å from the receptor. The potential fields emanating from the receptor are partially neutralized, the −350 kJ contour no longer spans the narrow groove and, as a result, the contours are compressed. Severe asymmetry is introduced into the fields between the base planes; this can be directly attributed to the unequal charge distribution in ethidium, the ethyl group being more positive and nearer to the phosphate than is the phenyl ring (§ 3). A less marked effect on the contour values is observed in the wide groove. The mapping section passes through the plane of the phenanthridinium ring and the 418 kJ contour lies close to the van der Waals surface for the molecule. Contour levels are closer to ethidium at the leading edge than on the trailing side. The local minimum along the flight path has shifted from the narrow groove towards the helix axis and now lies between the bases. A hidden line suppression profile is drawn in figure 6*b*, plate 6. This time the observer shares the 'receptor's-eye view' of the fields perturbed by the incoming drug molecule. Significant potential wells can only be seen on the narrow groove side, where there is a precipitous fall of potential, from ethidium to the phosphate wells. Inequalities in field structure between the base planes are clearly visible.

Movement of ethidium in to the 6 Å position (figure 5*c*) results in a further neutralization of fields between the bases, the potential having fallen, by 100 kJ, to −250 kJ. The 0 kJ contour has tightened round the trailing edge of ethidium. Electrostatic capture of ethidium by the receptor is distinctly seen, with negative electrostatic fields encircling the drug molecule.

Penetration of ethidium to 3 Å (figure 5*d*) diminishes the magnitude of the electrostatic fields to about −150 kJ mol^{-1} along the flight path. The local minimum is shifted to the wide groove side as the leading edge begins intercalative overlap into the inter-base region. A −50 kJ contour surrounds the trailing edge of the drug molecule. At 1 Å separation (figure 5*e*), there is a large overlap of the bases with the phenanthridinium ring, and the fields on either side of the mini-helix are both approximately −100 kJ mol^{-1}. This must be near to the electrostatic equilibrium position, since the potential is now almost balanced on either side of ethidium. For the fully intercalated position (figure 5*f*) the fields in the narrow groove are more negative, which would suggest that the drug molecule has travelled beyond the point of the electrostatic minimum; this, however, may be a geometry artefact generated by our (dC-dG)·(dC-dG) structure.

To summarize the results from this section, the important observations are: (*a*) the electrostatic potentials generated by the receptor and drug molecule undergo mutual interaction so that the resultant field is perturbed; (*b*) electrostatic capture of the drug molecule takes place; (*c*) the local minimum along the flight path shifts as the drug moves in; (*d*) the effect of this shifting local minimum is to pull the attacking molecule into the receptor cleft so that good overlap of the atoms is obtained. Once van der Waals contact between the bases and the drug has been established, other molecular forces may operate to force the ligand to jockey for an equilibrium position in the receptor.

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6. *From which groove is intercalative attack most likely?*

A consideration of the electrostatic fields surrounding (dC-dG)·(dC-dG) (figure 5*a*) would suggest that there is an attractive electrostatic gradient towards a cation on each side of the helix axis. To chart these fields a proton was placed at the grid positions in the frame and the

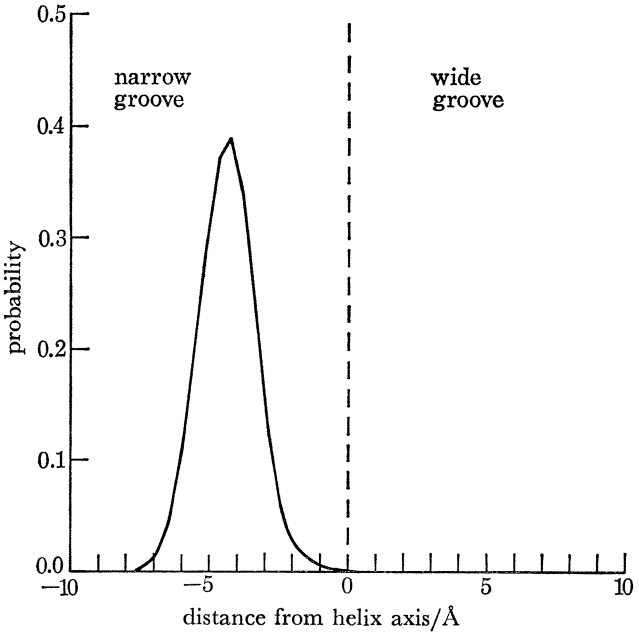

FIGURE 7. Boltzmann probability distribution for proton attack down the flight path. (Data derived from figure 5*a*.)

electrostatic contributions to the energy from all atoms in the molecule were summed. Therefore, by taking the energy values along the line of the flight path from figure 5*a*, a Boltzmann probability distribution could be constructed, integrated through a distance of 10 Å on either side of the helix axis and normalized to unity. This distribution is plotted in figure 7 and represents the probability of finding a proton at any position along the flight path. The probability becomes significant at about 8 Å in the narrow groove, is a maximum at 4–4.5 Å and then diminishes rapidly towards the helix axis, whereas in the wide groove there is no parallel distribution. There is, therefore, a single potential well in the narrow groove, capable of trapping a proton or other positively charged molecule. Moreover, this is found to lead into the region of inter-base overlap from the narrow groove side. Thus, in terms of probability, intercalative attack is more likely to occur from the narrow groove for this geometry of the receptor.

7. *Orientation effects imposed upon the docking drug molecule in the vicinity of the receptor*

The previous account illustrates the complex nature of the electrostatic component of the interaction in a single plane between receptor and drug molecules. In this section, the interaction energy differences, generated by rotating the ligand about its three Eulerian axes, are analysed. The PCILO method is used to calculate the total energy of the supermolecular system. This procedure has the advantage that polarization, delocalization and electron correlation effects are also considered. However, a substantial simplification of the receptor structure is required in this calculation for two reasons: (1) the total number of valence electrons that can

be handled in the computation is limited by arithmetic precision and the amount of computer main-core memory accessible; (2) the available forms of the PCILO program do not include d-orbital electrons, thus precluding phosphorus atoms. To alleviate these problems the deoxynucleosides are omitted and the phosphate groups replaced by formate anions with their oxygen atoms aligned to mimic the non-ester-linked phosphate oxygens. This drastic approximation of the receptor structure reduces the problem to a study of the rotation of the drug molecule in an electrostatic field which is derived solely from the anionic charges. Attack of ethidium on this structure is investigated from one side only (the narrow groove side, see section 6 and

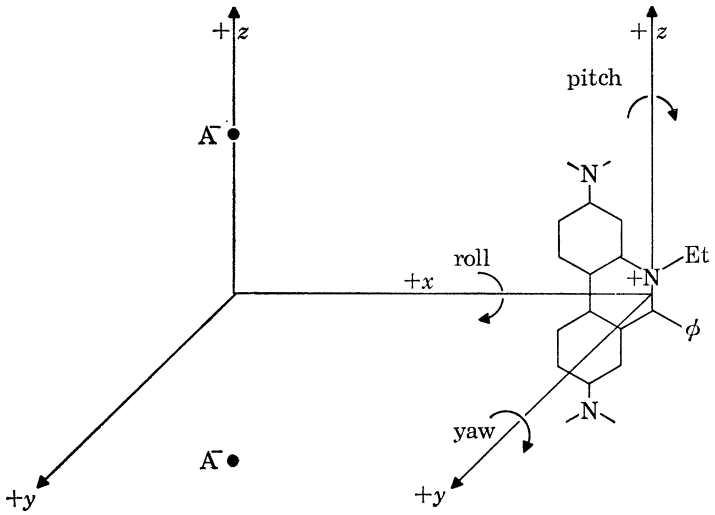

FIGURE 8. Definitions for rotation of the drug molecule about the Eulerian axes with respect to the Cartesian coordinate system of the receptor model. Roll rotations occur round the x-axis, yaw rotations round the y-axis and pitch rotations round the z-axis of the drug coordinate system. The origin of this system is the midpoint of the C=N bond of the phenanthridine. The position of the quaternary nitrogen atom and the direction of the ethyl group (Et), with respect to the drawn axes, define the orientation of the drug.

Initial conditions for rotations:

Roll = 0°:
quaternary N on negative z-axis,
Et has negative z and negative x coordinates,
quaternary N is rotated clockwise when viewed along $x \rightarrow$ receptor.
This sets yaw angle = 90° and pitch angle = 0°.

Yaw = 0°:
quaternary N on positive x-axis,
Et has positive x and positive z coordinates,
quaternary N is rotated clockwise viewed along $y \rightarrow$ origin.
This sets pitch angle = 0° and roll angle = 180°.

Pitch = 0°:
quaternary N on negative z-axis,
Et has negative z and negative x coordinates,
quaternary N is rotated clockwise viewed along $z \rightarrow$ origin.
This set yaw angle = 90° and roll angle = 0°.

figure 7) so that the drug molecule approaches the receptor along the flight path. The drug is allowed to rotate freely about two of its three major axes, such that it may roll and yaw along the translational axis, and a more restricted set of calculations are performed on pitching motions of the drug molecule. Figure 8 describes the definitions of roll, yaw, pitch and shows the initial positions used. Calculations are conducted for both ethidium and its p-carboxyphenyl

derivative at 6 Å and 12 Å separation between the N=C bond of the drugs and the carbon–carbon vector of the formate groups (equivalent to the phosphorus–phosphorus vector of the dinucleotide). Computational costs prohibit simultaneous variation in roll and yaw orientational angles; consequently, energy values are calculated at 30° intervals for rotation around one axis while an unique value for the other is maintained. Energy plots produced in this way are also converted to Boltzmann probability distributions. These add considerable clarity to interpretation of complex energy diagrams, since they indicate the relative populations of the rotating ligands.

(*a*) *Roll rotation effects*

The initial geometry is constructed with the phenanthridine aligned suitably for entry into the intercalation site, i.e., the yaw angle is set to 90°, so that a line joining the ethidium amino groups is parallel to a similar line drawn between the formate carbon atoms. In addition, the pitch angle is set to 0°, so that the phenyl and ethyl groups point away from the receptor. The roll rotation angle is defined as the angle between the plane of the phenanthridine ring and the plane joining the formate carbon atoms to the mid-point of the C=N bond of ethidium (see figure 8). However, this latter plane is slightly inclined with respect to the intercalation plane, and makes an angle of approximately 5° to it; this should be remembered when one considers the following rotational energy plots. Thus, a roll rotation angle of 0° or 180° signifies that the plane of the phenanthridine ring is aligned along the formate carbon–carbon vector and is rotated by approximately 5° with respect to the intercalation plane.

Figure 9*a* shows the variation in PCILO third order energy for the ethidium–formate complex (compared to the global maximum, which is set to 0 kJ mol^{-1}) as the drug molecule rotates about its *x*-axis (see figure 8), thereby revolving the plane of the phenanthridine ring around the translational axis. It can be seen that at 12 Å separation the total difference in conformational energy is small (no more than 12 kJ mol^{-1}) and that there are two broad minima situated around 0 and around 180°. Figure 9*c* shows the probability distribution obtained from this energy plot and reveals that there are no populations of molecules having roll angles of 60–120° and 240–300°. The rest of the rotational space is reasonably well populated, with orientation peaks at 30, 150, 210 and 330°, although none of these is particularly dominant. Hence, at 12 Å approximately two-thirds of the rotational space is occupied and the phenanthridine ring does not lie at right angles to the intercalation plane; thus, it is already partially oriented in the correct roll angle for docking. Furthermore, figures 9*a* and 9*c* indicate that very distinct preferences in orientation can be determined by quite small energy differences (e.g., 10–12 kJ mol^{-1}).

On approaching the receptor to 6 Å, there are now extremely large variations in roll orientation energies (figure 9*a*). The difference between the most and least favoured is 56 kJ mol^{-1}. The shape of the energy curve is considerably smoother and is almost symmetrical about the 180° value, with large energy minima at 0 and 180° and maxima at 90 and 270°. As would be expected from these large energy differences, the probability distribution (figure 9*c*) is totally dominated by just two configurations centred on $0 \pm 12°$ and $180 \pm 12°$ with orientations between 15–165° and 195–345° unpopulated. The peak probabilities are much larger; their distribution is narrower than at 12 Å and they are almost of equal probability, with a slight preference for 0°. Thus, as ethidium approaches its receptor, the drug molecule experiences considerable orienting effects. These cause the chromophore to rotate into the planes of the

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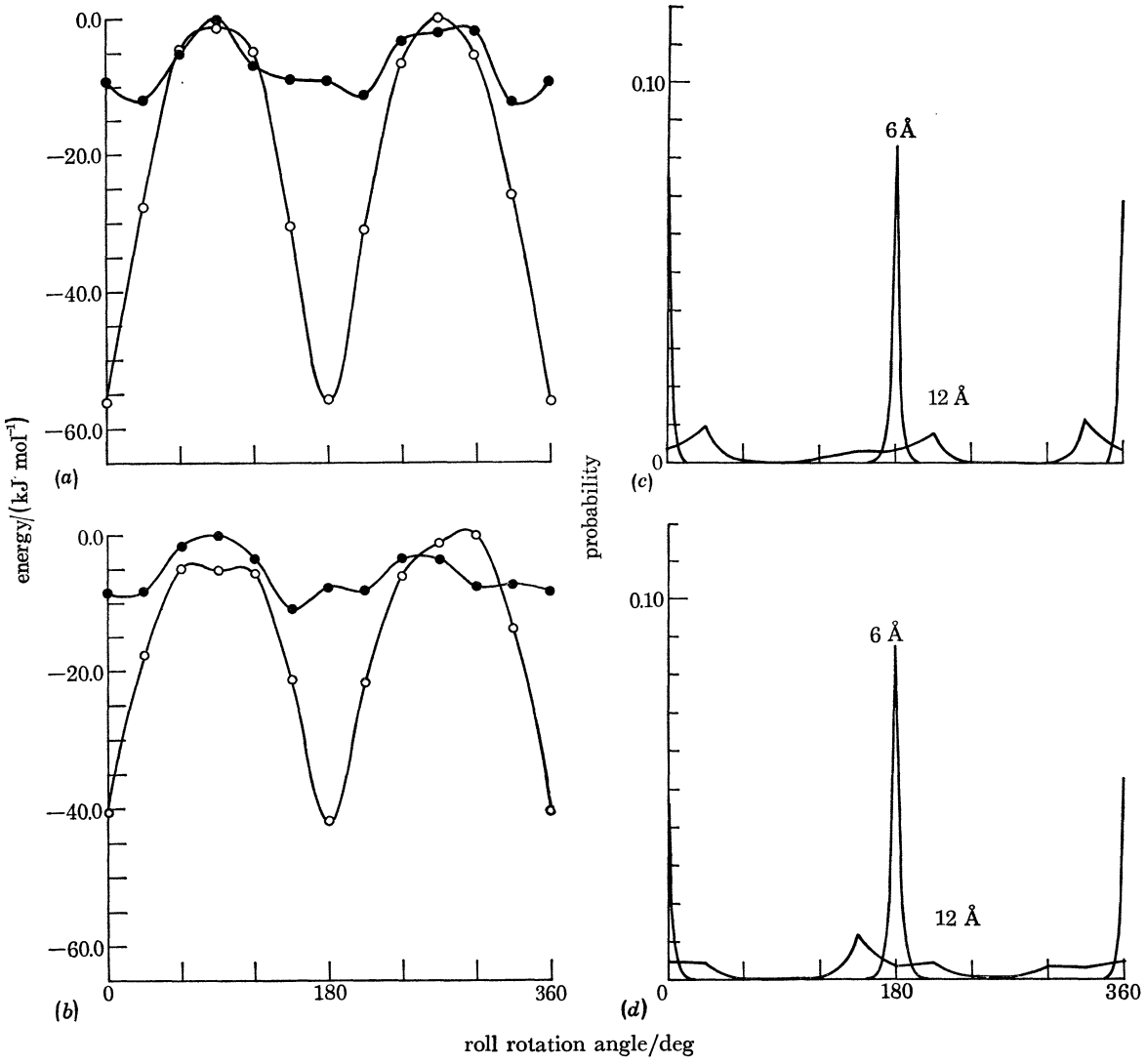

FIGURE 9. Energy and probability distributions for roll rotations of ethidium and *p*-carboxyphenylethidium: (*a*) ethidium roll energies at 12 Å (●) and 6 Å (○) separation from the receptor; (*b*) carboxyphenylethidium roll energies at 12° (●) and 6 Å (○) separation from the receptor; (*c*) data from figure 9*a* converted to a Boltzmann probability distribution for ethidium; (*d*) data from figure 9*b* converted to a Boltzmann probability distribution for carboxyphenylethidium.

formate–formate vector, which effectively aligns it to within 5° of the intercalation plane. The electrostatic field pictures of the receptor (dC-dG)·(dC-dG) (figure 4) show that the bases cause the fields to twist between the base planes as they pass through the intercalation site. The formate model of the receptor cannot simulate this effect, but it seems reasonable to argue, from a combination of the above calculations and field pictures, that ethidium is brought to the dinucleotide aligned with the phosphate–phosphate vector. The fields modified by the bases then rotate the ligand through the last 5° into the intercalation plane. Finally, the probability distributions indicate that, as the drug approaches, its rotational degree of freedom is severely curtailed and that, while it can attack in one of two configurations, rotation close to the docking site would be extremely unlikely because of the inhibitory effects of the 56 kJ mol^{-1} energy barrier.

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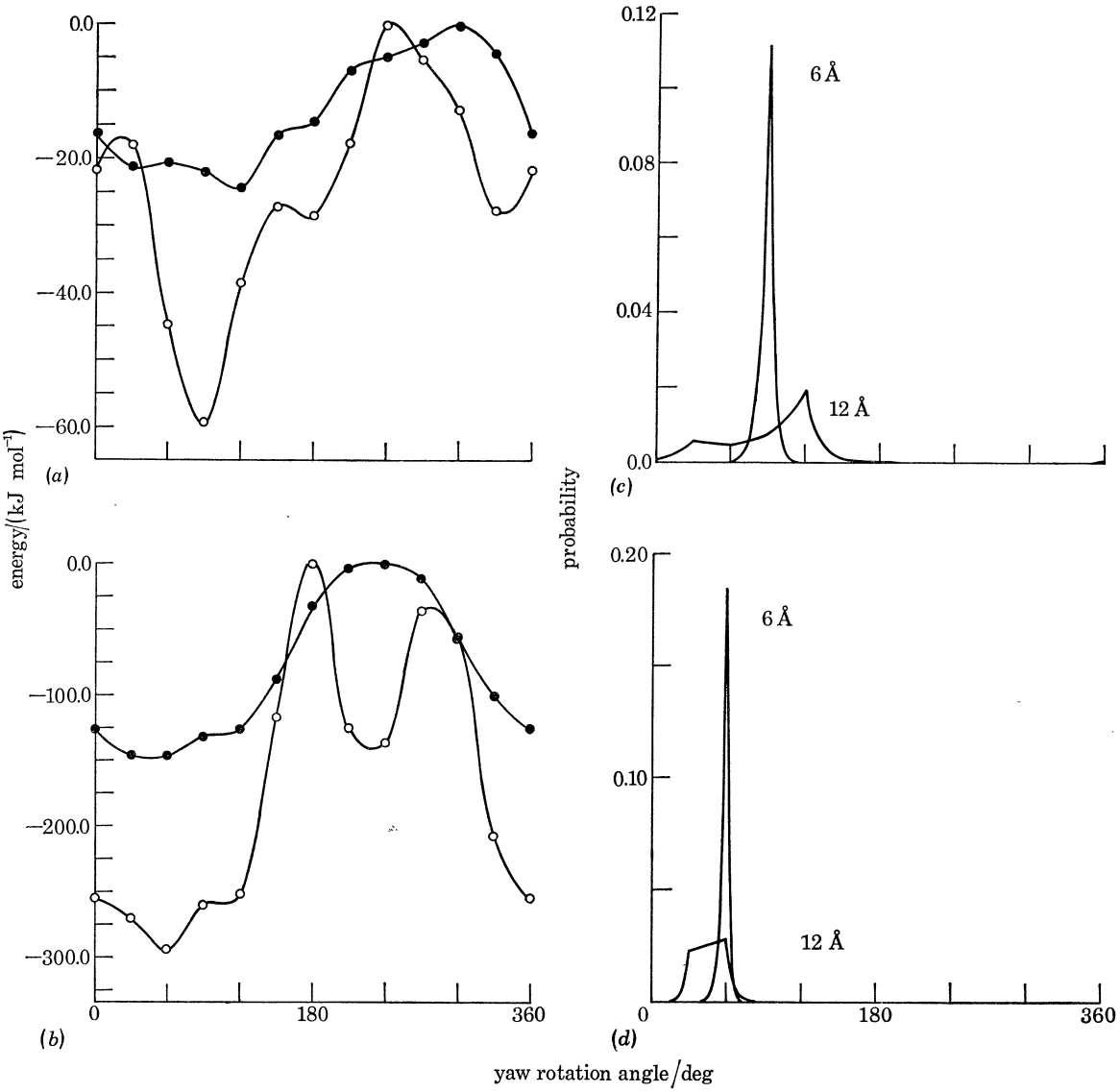

FIGURE 10. Energy and probability distribution for yaw rotations of ethidium and *p*-carboxyphenylethidium: (*a*) ethidium yaw energies at 12 Å (●) and 6 Å (○) separation from the receptor; (*b*) carboxyphenylethidium yaw energies at 12° (●) and 6 Å (○) separation from the receptor; (*c*) data from figure 10*a* converted to a Boltzmann probability distribution for ethidium; (*d*) data from figure 10*b* converted to a Boltzmann probability distribution for carboxyphenylethidium.

The effects of addition of an ionized carboxyl group to the phenyl ring of the drug molecule on the roll rotation energies are given in figure 9*b*. At 12 Å from the receptor, the zwitterion shows favourable conformational energies around 0 and 180°, in a manner very similar to that of the parent compound. Indeed, the values of the roll rotation energies for the two molecules at this distance are comparable. Comparison of the probabilities derived from these energies (figure 9*d*) with those for ethidium (figure 9*c*) indicates that, at moderate distances from the receptor, the electrostatic effects on roll orientations of the negatively charged 'tail' are minimal. However, on approach to 6 Å, slight changes occur in the energy profile for the zwitterion (figure 9*b*), compared with ethidium. The magnitude of the total energy difference is now

smaller (figure 9*a*), i.e. 42 kJ mol^{-1}, compared with 56 kJ mol^{-1}. The probability distribution at this distance (figure 9*d*) reveals that the most favoured conformation is that with the roll angle at 180°, compared to 0° for the parent drug.

(*b*) *Yaw rotation effects*

Yaw effects occuring in the vicinity of the intercalation plane have been examined. The roll rotation angle is set to 180° and the pitch to 0°, which flips ethidium over, compared to its (slightly) preferred roll rotation of 0°. This configuration is a little more favourable for the zwitterion and is adopted for ethidium as well to permit direct comparison of results. The yawing motion is achieved by rotation of the phenanthridine ring about the *y*-axis (figure 8) and results in rotation of the phenyl and ethyl groups towards the formate anions.

Figure 10*a* reveals that yawing ethidium at 12 Å from the receptor produces an energy profile that is considerably more complex than that obtained for roll rotations at this distance (cf. figure 9*a*). In particular, the conformational energy differences are much greater (25 kJ mol^{-1}) and do not show the pseudo-symmetrical nature of the roll rotations. Presumably, this asymmetrical character reflects the charge distribution of the ethyl and phenyl groups on the drug molecule. The lowest energy regions occur between 0 and 150°, with a broad minimum situated at 120°. This range of conformations has the phenyl ring positioned between the formates and distal to the receptor. The most unfavourable conformations occur at yaw angles in the region of 300°, whereupon the phenyl ring is proximal to the receptor and in the vicinity of one formate anion; the ethyl group lies towards the other formate. The probability distribution (figure 10*c*) demonstrates that angles between 0 and 180° are well populated; the most probable conformation occurs at 120°. Therefore, once more, the attacking ligand is showing preferences for defined orientations as it approaches the receptor.

At 6 Å, the energy differences are much larger, with the most favoured orientation now being at 90°. In this position, the chromophore is correctly aligned for intercalation and the substituents are directed away from the receptor (figure 10*a*). The least favoured orientation is at 240°, a conformation which has the phenyl ring pointing between the formates and the ethyl group juxtaposed to one of the formate anions. The probability distribution (figure 10*c*) clearly indicates the total dominance of the single orientation at 90 ± 30°. Thus, on approaching the receptor, the most favourable orientation for the drug molecule shifts from 120 to 90°, which brings the drug into a suitable conformation for complex formation. The allowable range of orientations closer to the target is again severely restricted (figure 10*c*).

In contrast to the small effects of the carboxyl group on roll rotations, the influence on the yaw energies is extremely large. Figure 10*b* illustrates these energy variations at 12 Å, where it can be seen that the minimum energy occurs at 30–60°; the latter conformation has the carboxyphenyl group aligned along the flight path. The low energy region between 0 and 120° occurs when the carboxyphenyl group is positioned distal to the receptor, but, as soon as it swings towards the formates, the energy rises by approximately 146 kJ mol^{-1}. The energy profile is smooth and symmetrical between 120 and 360°. This may be attributed to the rotation of the drug in the region of the flat electrostatic field of the intercalative plane (see figures 5*b* and 6*a*). The most unfavourable conformation is at 240°, where the carboxyphenyl group again lies on the flight path and is proximal to the receptor. The probability distribution (figure 10*d*) reveals that only angles between 18 and 78° are populated, with the majority of molecules lying in the range 30–60°. This is a considerably different distribution to that

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obtained for ethidium and would appear to result from the electrostatic field interactions of the carboxyphenyl and formate groups.

The conformational energy differences are increased on translation of the zwitterion to within 6 Å of the receptor (figure 10*b*): they now span a range of 293 kJ mol^{-1}. There is a global minimum at 60°, where the carboxyphenyl group points away from the formates. Unlike ethidium, there is no clear evidence from the energy plots for rotation of this derivative on approach to the receptor. The energy profile between 120 and 360° is remarkably different at 6Å from that at 12 Å. The smooth symmetrical character found at the greater distance is lost and replaced by two large peaks separated by a trough; this trough is coincident, at 240°, with the peak observed at 12 Å. On rotation from 120–180°, the carboxyphenyl group sweeps into the deep electrostatic potential well of the formate group. The shape and position of this well may be visualized for the phosphate group of the dinucleotide in figure 6*a*. With the carboxylate group adjacent to this formate, very unfavourable interactions occur and the conformational energy rises by 251 kJ mol^{-1}. As the carboxyphenyl group rotates in front of the receptor, it moves from the local repulsive region into an area of less repulsion, until it reaches an angle of 240°, where the carboxylate group is positioned equally between the formates; here, the energy is approximately 146 kJ mol^{-1} more favourable. On further rotation to 270° the carboxyphenyl group moves into the vicinity of the second formate and the ethyl group lies facing the first; this time the energy rises by 84 kJ mol^{-1}. These large conformational energy differences are reflected in a very restricted range, around 60 ± 18°, of probable orientations for the docking drug (figure 10*d*). Thus, as the drug approaches the receptor, the presentable configurations change from a range of 30–60° to a far more constrained rotation centred on 60°. Consequently, there is no fundamental change in attack orientation, but more of a 'tightening-up' of allowable oscillations.

(*c*) *Pitching effects*

The combination of roll and yaw configurations studied also provides information about pitching of the phenanthridine chromophore. Pitching describes the motion of the drug molecule when it is rotated round the *z*-axis (which is orthogonal to the roll and yaw rotation axes), so that the phenyl ring is translated from its position pointing away from the intercalation site (pitch angle 0°) to one in which it is proximal to the receptor (pitch angle 180°, see figure 8). Performance of this motion for ethidium at 12 Å (pitch angle 0–180°) results in a reduction of the interaction energy by +7.1 kJ mol^{-1}, which increases to a value of +54.3 kJ mol^{-1} at 6 Å. Thus, even at 12 Å, the pitching motion is unfavourable and, at 6 Å, such manoeuvres would be extremely unlikely. The electrostatic effects attributable to the carboxylate group are very well demonstrated in the pitching rotation of the zwitterion, since the relevant energy terms become +129.1 kJ mol^{-1} and +258.1 kJ mol^{-1} at 12 Å and 6 Å, respectively, for this derivative. Therefore, it is effectively impossible for the carboxyphenyl group to adopt a configuration in which it would 'lead' the attack on the intercalation site.

8. *Partition of the interaction energy for the docking manoeuvre*

The total energy calculated by the PCILO method can be split into various contributions that may be identified with definite physical concepts (Malrieu 1977). This provides the opportunity of establishing which, if any, are the major components of an energy change

produced when a molecular system is perturbed. The zero order energy is decomposed into one- and two-bond terms: this decomposition appears, first, as a sum of one-bond energies involving a kinetic energy part plus the electrostatic interactions between the electrons and bond-nuclear charges of each bond, and, secondly, as a sum of two-bond energies representing the electrostatic interactions between the charge distributions of the pairs of bonds. It is, therefore, a simulation of electrostatic and short range repulsion energies. Second order contributions are derived from bond-polarization corrections, the delocalization energy, intra-bond correlation energy and inter-bond correlation energy. Third order contributions are obtained from the singly and doubly excited determinants of the first order wave function and, in general, produce only small corrections to the final total energy.

In the previous section, the roll and yaw components of a docking manoeuvre for ethidium and carboxyphenylethidium in an anionic field were examined and marked preferences for particular relative orientations were observed. A question that can be asked is: what are the underlying energetic terms that determine the energy differences between docking positions, for example, to what extent do localization and delocalization effects influence the interaction energy? Energy contributions for the most and least favourable roll and yaw orientations at 6 Å and the energy partition for translation from 12 to 6 Å for the most favourable roll rotation are given in table 2.

(*a*) *Roll*

For ethidium, the roll rotation barrier is 56 kJ mol^{-1} and, for carboxyphenylethidium, 41.4 kJ mol^{-1} (table 2). With both drug molecules the major contribution to the energy barrier is from the electrostatic and short range repulsion energies in the zero order term, amounting to 45.2 % of the total energy difference for ethidium and 42.3 % for carboxyphenylethidium. Second order contributions, however, are also very important, with the delocalization energy the dominant term, amounting to 37.7–40.9 % of the roll rotation barriers. Third order energy corrections are very small and are about 11 % in both instances. Thus, the roll rotation barrier is not purely an electrostatic phenomenon of a multipole interaction type, but has a significant component from electron reorganization in the interacting molecules.

(*b*) *Yaw*

Whereas for roll rotation the pattern of energy contributions for ethidium and carboxyphenylethidium was essentially similar, the energy partition for the yaw rotation barriers is very different (table 2). The total yaw rotation barrier for ethidium is 59.4 kJ mol^{-1} and for carboxyphenylethidium is 294.7 kJ mol^{-1}. With ethidium, the dominant energy contribution arises from the delocalization energy and amounts to 47.3 % of the total yaw rotation barrier. The zero order energy is 40.4 % of the total barrier. On the other hand, with carboxyphenylethidium, 86.3 % of the yaw rotation barrier is contained in the zero order term, with only tiny contributions from delocalization and third order terms (5.5–6.9 % in each instance). Therefore, with carboxyphenylethidium yaw rotations, the electrostatic and short range repulsion terms predominate when the molecule is rotated into an unfavourable position, the reason for this being that the anionic charge of the drug carboxyl group, unlike the charge of the quaternary moiety, is not substantially delocalized into the rest of the molecule (see § 3). Hence, when the molecule is rotated so that the carboxyl group points towards a

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TABLE 2. PCILO SUPERMOLECULAR ENERGY CONTRIBUTIONS (kJ mol^{-1}) TO ROLL, YAW AND TRANSLATIONAL MOTIONS OF ETHIDIUM AND CARBOXYPHENYLETHIDIUM IN THE SIMULATED RECEPTOR FIELD

		ethidium			carboxyphenylethidium		
energy contribution		roll rotation barrier at 6 Å 0–270°	yaw rotation barrier at 6 Å 90–240°	translational energy gain 12–6 Å	roll rotation barrier at 6 Å 180–300°	yaw rotation barrier at 6 Å 60–180°	translational energy gain 12–6 Å
E_0		29.01	25.87	−92.50	19.86	264.39	−32.40
polarization energy	E_2	0.00	0.00	0.00	0.00	0.00	0.00
delocalization	E_2	24.20	30.26	−22.46	19.19	17.10	−18.81
intra-bond correlation	E_2	−2.38	−1.50	2.09	−1.80	−3.51	1.63
inter-bond correlation	E_2	−1.55	−0.75	1.17	−0.88	−0.33	0.63
E_3		6.90	5.64	−8.99	5.06	16.97	−6.02
$\|E_3\|$		7.06	5.64	9.03	5.23	21.07	6.10
E_{tot}		56.18	59.52	−123.69	41.42	294.61	−54.97
$\|E_{tot}\|$		64.20	64.04	130.25	46.94	306.39	59.57

E_n is the nth order energy contribution and E_{tot}, the total energy of the molecule.
The moduli of E_3 and E_{tot} are given for calculation of percentage differences used in the text.

receptor-generated anionic field, electrostatic repulsion overrides all other contributary terms to the total interaction energy.

(*c*) *Translation*

The energy gained by moving ethidium from 12 to 6 Å from the formate carbon–carbon vector is −123.7 kJ mol^{-1}, more than double the energy gain for the corresponding translation with carboxyphenylethidium (table 2). With both molecules, the increase in translational energy is predominantly in the zero order term and is essentially of electrostatic origin: for ethidium, 71 % of the total energy is electrostatic and, for carboxyphenylethidium, 54.4 %. The delocalization energy is significant for both drugs, amounting to 19.5 % and 31.6 %, respectively, for ethidium and carboxyphenylethidium.

The results for the partition of interaction energy in the docking manoeuvres can be summarized. The polarization energy never amounts to more than 0.01 kJ mol^{-1}. Correlation energy differences (intra- and inter-bond) are small, being less than 3.8 kJ mol^{-1}. The delocalization term is predominant in the second order energy and significantly affects the total energy differences by up to 30.3 kJ mol^{-1}. Third order energy corrections do not add up to more than 11 % of total energy differences. The zero order energy forms a substantial component of the energy barrier (greater than 40 %). Therefore, one can answer the questions, posed previously, about the origin of the conformational preference in the docking manoeuvre. In each case considered, between 82 and 92 % of the total energy barrier could be accounted for by changes in just two energy contributions, namely the zero order level and the delocalization energy. Having distinguished the major components of the interaction energy, we can illustrate their meaning in two simple ways. First, dipole moment changes can be correlated with the electrostatic component and, secondly, electron reorganization can be monitored to show the delocalization phenomenon.

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(*d*) *Dipole moments*

Part of the electrostatic contribution to the final energy differences in the docking manoeuvre can be analysed in terms of the dipole moments of the receptor and drug molecule. The PCILO method calculates the total dipole moment, μ, as a sum of two contributions:

$$\mu = \mu_{ch} + \mu_{hy},$$

where the first contribution, μ_{ch}, is obtained by vector summation of the net charges located at the nuclear positions; the second term is the atomic hybridization dipole moment and takes account of the fact that the centroid of charge does not necessarily coincide with the centre of nuclear charge. It was found that roll rotations alter the total dipole moment of the supermolecular system by only a small amount (< 1D) for each of the drugs, whereas for yaw rotations there is a large change (9D for ethidium, 61D for carboxyphenylethidium). The reason for this can be discerned by reference to the direction and magnitude of the ligand dipoles shown in figure 3; the dipole for each drug lies almost entirely in the plane of the heteroaromatic ring. The potential field generated by the receptor anionic charges is a vector field. Rotation round the roll axis causes the drug dipole to move perpendicular to the lines of force with no sign change in vector summation. On the other hand, rotation round the yaw axis causes the dipole to rotate parallel to the lines of force; therefore, vector addition with a sign change is very different in the parallel and anti-parallel alignment situations. The preferred configuration occurs when the vector sum of dipole moments in the associating molecules is a minimum, that is, when the resultant dipole between each species shows a maximum cancelling effect in the vector addition.

(*e*) *Electron delocalization*

Perhaps one way of understanding the delocalization contribution is to examine the localization of charge on the two amino group nitrogen atoms of each drug molecule (table 3). Of course, there is no reason why other atoms should not be studied in this way, but a careful analysis of them all would be impracticable; therefore, the atoms showing the greatest redistribution of charge have been selected as indicators of delocalization. In the isolated state there is little difference in the charges of both atoms for each molecule (approximately 2–5 m*e*). On translation to 12 Å, this difference widens to 25 m*e* for the sp^2 nitrogen atoms of both drugs and increases by no more than 1 m*e* for their sp^3-hybridized atoms. Further penetration of the receptor fields to 6 Å augments these charges by another 11–12 m*e* and 3–4 m*e* for the sp^2 and sp^3 atoms, respectively. Charge differences on the sp^2 atoms are greater than on the sp^3 nitrogens in roll and yaw rotations with both drugs. Yaw, rather than roll, rotations have the more pronounced effect on sp^2 charges, but the converse is true for sp^3-hybridized atoms. The greater fluidity of electronic charge centred on the sp^2-hybridized nitrogens results from the fact that their lone-pair electrons reside in π-bonding p-orbitals that are resonant with the heteroaromatic system. On the other hand, the lone–pair of the sp^3-hybridized atoms occupy a σ-orbital that lies on one side only of the phenanthridinium plane and is not orthogonal to it. Thus, we have a simple indication that electron shifts accompany the orientation movements of these two drug molecules as they roll and yaw in a simulated anionic field representing that generated by the receptor, and that this delocalization is a very significant contribution to the total rotational barriers.

TABLE 3. NET ELECTRON CHARGES (e) ON THE AMINO GROUP NITROGEN ATOMS OF ETHIDIUM AND CARBOXYPHENYLETHIDIUM AT ENERGY EXTREMES OF THE DOCKING MANOEUVRE

(Roll and yaw are at 6 Å.)

	ethidium		carboxyphenylethidium	
energy extreme	sp^2 hybridized	sp^3 hybridized	sp^2 hybridized	sp^3 hybridized
isolated molecule	−0.1903	−0.1921	−0.1985	−0.1937
roll favourable	−0.1530	−0.1877	−0.1623	−0.1902
roll unfavourable	−0.1697	−0.1932	−0.1756	−0.1982
yaw favourable	−0.1530	−0.1877	−0.1649	−0.1957
yaw unfavourable	−0.1740	−0.1864	−0.1842	−0.1925
translation 6 Å	−0.1530	−0.1877	−0.1623	−0.1902
translation 12 Å	−0.1652	−0.1913	−0.1737	−0.1936

9. *Orbital energy changes in the docking of ethidium*

The interaction energy, W, for two reactant molecules can be expressed by the equation (Fukui 1975):

$$W = E_Q + E_K - D,$$

where E_Q is the Coulomb interaction term arising from the net charge on each atom of the two molecules and is significant for polar molecules over long separation distances, E_K is the exchange interaction term and operates predominantly over short distances, D is the stabilization energy due to electron delocalization interaction and is dependent on the energy levels of the occupied and unoccupied orbitals. Therefore, in the interaction of charged species, the E_Q term plays a major role over large distances, whereas, upon close approach, the effect of D

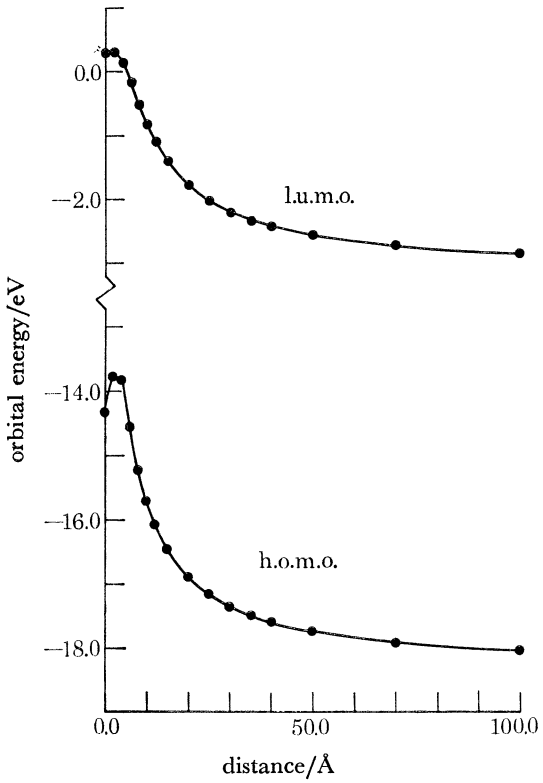

FIGURE 11. Energy levels for the highest occupied and lowest unoccupied bond molecular orbitals of ethidium as it approaches the receptor. For isolated ethidium h.o.m.o. = −18.4836 eV and l.u.m.o. = −3.1331 eV.

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increases and, in the region of orbital overlap, the highest occupied molecular orbital (h.o.m.o.) and the lowest unoccupied molecular orbital (l.u.m.o.) are of paramount importance. In the previous section we have already scrutinized changes in E_Q and D for rotational orientations. This section is devoted to examination of the variations in h.o.m.o. and l.u.m.o. for translational movement.

The perturbation of h.o.m.o. and l.u.m.o. for ethidium moving into an anionic field has been analysed with use of the diformate–ethidium model. In ethidium, h.o.m.o. is found to be the lone–pair of the sp^2-hybridized 3-amino nitrogen atom, l.u.m.o. is the π-antibonding orbital in the C=N bond of the phenanthridinium ring. The orbital energies are: h.o.m.o., -18.48 eV; and l.u.m.o., -3.13 eV. Thus, ethidium is a good electron acceptor and a bad electron donor, as one would expect for a positively charged molecule. Figure 11 illustrates the shifting frontier orbital densities of ethidium travelling along the flight path and in the glide plane towards the receptor. Profound changes in h.o.m.o. and l.u.m.o. occur even at quite large separation distances from the anionic sites. H.o.m.o. changes by 1 eV on movement from 100 to 20 Å separation and then rapidly rises a further 3 eV when brought to 2 Å. The effect for l.u.m.o. is similar, with a gradual swing of about 3 eV towards a positive orbital energy as ethidium approaches the receptor. These changes in h.o.m.o. and l.u.m.o. occur before any charge transfer is perceived. It has already been noted that the lone-pair electrons of the guanine amino nitrogen atoms are directionally located normal to the base plane and immediately above the position where the l.u.m.o. orbital of ethidium is found in the crystal structure of ((iodo–C)–G)·((iodo–C)–G)–ethidium (Jain *et al.* 1977). A possible contributary factor to the stabilization of ethidium in the drug–receptor complex may be electron donation from this guanine nitrogen atom into the l.u.m.o. orbital of ethidium. The observations of shifting orbital densities and electron rearrangements for ethidium indicate that it may be profitable to investigate the stability of the drug–receptor complex between ethidium and the base pairs, within the framework of the theory of orientation and stereoselection proposed by Fukui (1975).

General discussion

In this paper we have attempted to develop a rationale for a quantum mechanical analysis by approximate molecular orbital theory of those factors that determine the orientation of a drug before complex formation with a receptor. The example of intercalation into a dinucleotide is a simple illustration of the events taking place at an active site; the investigation is made considerably easier by the fact that a small number of atoms (124) form the active receptor unit. Conformational variations in the drug molecule are also limited, since the active moiety is the heteroaromatic phenanthridinium ring. By use of these semi-empirical quantum mechanical methods it is possible to study the electron distribution in the receptor and the drug molecule, the electrostatic fields generated round the receptor active site and the drug, the perturbation of the electrostatic fields as the two molecules approach each other, the probability of the best line of attack, the orientation effects of roll, yaw and pitch in a docking manoeuvre, the modification of drug structure to analyse design features for new drugs, the elucidation of major energy contributions that determine orientation preferences and the fluctuations in molecular orbital energies on close approach of a drug to its receptor. There is no reason why, when other receptor structures become available, similar computations should not be performed on further drug–receptor systems.

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Even with a small drug receptor a considerable simplification in methodology is needed, since it is not yet possible to do full approximate molecular oribital calculations on 168 atoms that were involved in the ethidium–(dC-dG)·(dC-dG) study reported here. Approximations have been introduced at two steps. First, the overlapping fragment method was used to determine the electron distribution which was needed for the electrostatic field calculations. Secondly, the PCILO program is not parameterized to deal with penta-coordinated atoms with 3d-orbital involvement in the wave function. Therefore, in the docking manoeuvre calculations, a gross simplification was made by substitution of a formate anion with the O=C—O$^-$ orientation set to that of the O=P—O$^-$ geometry. The object of the work has been to try to understand the principles of the docking manoeuvre rather than to achieve an 'accurate' solution. This technique of reduction of a problem to its basic elements is common in the application of quantum mechanics to biological problems, where one is handicapped by limited computing facilities. Thus, with larger receptor molecules, it may be necessary to use empirical methods in a preliminary procedure to discover more probable conformations, before any quantum mechanical analysis. This method has been used effectively by Warshel & Levitt (1976) in their study of lysozyme. The problem of elucidation of the docking manoeuvre is exacerbated by highly flexible torsion links in the drug molecule.

Quantum mechanical methods are currently employed extensively in the study of biomolecular problems (Lowdin 1974, 1975; Lowdin & Sabin 1976, 1977; Bergmann & Pullman 1974). In the great majority of examples investigated, the molecular wave functions are analysed for events occurring in a vacuum, so that the influence of the natural environment, for example, aqueous solution, is not included. The effect of solvent on quantum mechanical calculations is complicated by the problem of structural inhomogeneity in the vicinity of the ion. Three regions of solvent can be recognized: (*a*) immobile water molecules vicinal to the ion, (*b*) a disordered zone near to that structure, resulting from opposing forces between the ion and the distant water molecules and (*c*) water structure of the bulk solvent (Beveridge & Schnuelle 1975). Quantum mechanical studies on the hydration of nucleic acid components indicate that, in the region of the phosphate group, water molecules may be arranged in two hydration shells, with possible residual organization in a third layer extending about 10 Å from the phosphate atoms (Pullman *et al.* 1975; Clementi & Corongiu 1979). Hydration regions have also been located round nucleic acid bases (Port & Pullman, 1973; Pullman & Perahia 1978; Pullman *et al.* 1979); however, water binding to ethidium has not been studied. At distances where hydration shells are encountered, the dielectric shielding of the electrostatic potential may differ significantly from that of the bulk solvent, for example, Noyes (1962) has suggested that the local dielectric constant vicinal to an ion in solution is small. Similarly, Hopfinger (1977) argues that the dielectric medium between two solute species should have a shielding constant close to one when the interaction distance is less than, or near to, the diameter of a solute molecule. The problem is therefore one of distinguishing between the importance of the dielectric and ion hydration effects. Various attempts to estimate the influence of a dielectric continuum on charge distributions and conformational energies have been made (McCreery *et al.* 1976*a*, *b*; Beveridge & Schnuelle 1975); change of the dielectric exerted little difference on the relative conformational energies in the absence of solvent hydrogen bonding. Pullman *et al.* (1978) have studied the interaction through water between phosphate anion and sodium cation. With the hydrated structures separated by about 10 Å, charge transfer between the ions, accompanied by polarization of the water molecules, was demonstrated, whereas, in the

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absence of the hydration layers, no charge transfer could be detected. The existence of appreciable energies of interaction between the different hydrated complexes suggested many possible states for the phosphate–sodium–water system. It may thus be misleading simply to include bulk dielectric effects into calculations for distances of about one molecular diameter or shorter (Buckingham 1978); more important contributions would be expected from the supermolecule approach by hydration of the receptor and drug molecules. However, an increase in the dielectric constant would diminish the interaction and rotation energies, with a consequent broadening of the peaks in the probability distributions. In this initial attempt at analysis of the docking manoeuvre, we have ignored completely any environmental effects, to simplify the calculations and assess the relative difference in quantum mechanical parameters in the interacting molecules *per se*, so that features of interest can be identified. All the computations have been performed by semi-empirical methods, i.e., experimental parameters are introduced to solve the wave equations and reduce the computing time. This method diminishes the accuracy of the calculations, but this is not so important where relative changes are examined. Similarly, the electrostatic field calculations by the vss approximation frequently underestimate the magnitude of potential wells (Giessner-Prettre & Pullman 1972); this must be remembered during analysis of the field pictures. Nevertheless, the procedure is useful for examination of the shapes of the potential fields around molecules.

The results of docking manoeuvre calculations described in this work fall into three categories: an analysis of the electrostatic fields surrounding the receptor and the approaching drug molecule; a description of receptor-induced ligand orientation (r.i.l.o.) effects; and a decomposition of the interaction energy as the molecules move relative to each other. These three findings and their possible importance to molecular pharmacology will now be discussed separately.

(*a*) *Electrostatic fields*

Molecular electrostatic potentials for a number of important drug molecules have appeared in the literature as contour maps in a single plane (Loew *et al.* 1974). However, the fields for a receptor site of a macromolecule and the perturbation of these fields by an incoming ligand are novel descriptions. The electrostatic fields emanating from a molecule are generated by the imbalance of residual electronic charge on the constituent atoms. Thus, at any point in space the potential field is the result of summation of the fields generated by each atom. Consider the contribution from a single atom. The electrostatic potential is created by the residual atomic charge and the effective screening parameter of the nuclear charge. The potential then decays with distance from the nucleus. The molecular electrostatic potentials are mapped by their interaction with a proton and, therefore, the resultant maps are really electrostatic interaction energy surfaces. It may be helpful to describe the properties of these electrostatic fields in simple terms to gain a better understanding of their significance for drug–receptor interaction. To describe these surface shapes fully is a four-dimensional problem and, thus, can only be examined effectively by taking a series of energy contour values and studying them sequentially. This was the reason for the choice of the -334 and -418 kJ mol^{-1} contours in figure 4 for the three-dimensional receptor. In these illustrations, only local potential wells with an energy minimum greater than -334 kJ mol^{-1} are observed; smaller local minima are missed. Local energy wells are seen near the phosphates and the guanine oxygen atom. The shapes of the fields are different for the chosen energy values because of the summation effect and decay of

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potential with distance; this is well illustrated for the -418 and the -334 kJ mol^{-1} surfaces in figure 4. The -418 kJ mol^{-1} surface is separated into two regions centred on the phosphate groups, whereas, at -334 kJ mol^{-1}, the surface encompasses the dinucleotide. A larger spatial volume is occupied by the regions generating high field strengths; this is well demonstrated in figure 4*d*, where the larger potential well is that found near the phosphate rather than that near the guanine oxygen atom. Another feature that can be shown simply in the hidden line profiles of figure 6 is the accelerating potential gradient as a well is approached. This gradient fluctuation is related to the constraints to rotation inflicted upon an incoming ligand.

By carefully scrutinizing the three-dimensional potential fields generated by a receptor, we can gain some idea of the expected docking properties of complementary drug molecules, since the long range interaction forces are predominantly electrostatic. For example, the phenanthridinium ring of ethidium has a roughly discus-shaped potential field emanating from its overall positive charge. An examination of figure 4*c* would suggest that a good initial guess for a docking calculation would be to line up the drug molecule so that the amino ends are embedded in each spherical electrostatic surface of the phosphates, rather than tangential to these surfaces; this supposition has been substantiated by the roll–rotation calculations. Similarly, the bulk of the electrostatic fields for the geometry used lie on the narrow groove side of the mini-helix; this suggests that this side would be more likely to be attacked by a positively charged ligand; probability calculations again supported this hypothesis. Therefore, with complex and asymmetric receptor sites this procedure of mapping the potential fields in three-dimensional space could provide a starting configuration for costly docking manoeuvre calculations.

Electrostatic field description of the active site of receptors would be a very useful approach to rational drug design, particularly for competitive antagonists. The method would allow calculation and display of the spatial disposition of local minima and shapes of the bulk fields. With this information it should be possible both to design a molecule with a complementary electrostatic shape and to use the 'electrostatic rudder' concept to steer the ligand into the best fit. The potential fields are constructed by interaction with a proton and, therefore, they are excellent diagnostic aids for locating wells for possible hydrogen bonding (Pullman & Berthod 1978; Perahia & Pullman 1978). Hence, with these electrostatic methods it should be possible to construct, rationally, high affinity and site-specific binding agents.

As the drug molecule approaches the receptor, the electrostatic fields are perturbed considerably and are the result of summation of the individual potential fields at each point. In the instance where two molecules are oppositely charged, there is partial neutralization of local potential fields and the volume enclosed by a potential surface contracts. An important observation to draw attention to in the example of ethidium and the dinucleotide is the fact that, although the net charge of the complex is reduced by 1*e*, the local potential fields in the region of the phosphates are reduced only by a small amount, from -760 to -635 kJ mol^{-1}. Therefore, even though the overall charge is halved, there is considerable potential left in the phosphate region, which could accommodate perhaps another ethidium molecule. This may account for weak secondary binding of ethidium to DNA at high binding ratios in low salt buffer solution (Waring 1965; LePecq & Paoletti 1967). The neutralization of electrostatic potential has occurred largely in the spatial region between the phosphate groups.

The field perturbation effects of the docking entry of ethidium into (dC-dG)·(dC-dG) show the interesting phenomenon of a shifting electrostatic minimum from one side of the helix

to the other, and, at the same time, the minimum is diminished until the fields on each side are approximately balanced. This ligand interaction is of the Fischer 'lock and key' type with the conformations frozen. Shifting local minima may well be important in a more complex interaction of the 'zipper mechanism' type where the first event is the recognition of a ligand nucleation site by the receptive macromolecule. A shifting energy well, created by receptor interaction with a nucleation site, may provide enough electrostatic potential to reduce the conformational barrier for an adjacent rotatable group to orientate itself towards the created local minimum. This event may bring that group into binding contact with the macromolecule and so propagate ligand binding stepwise. Therefore, we have the possibility that zipper binding might be driven by a sequential r.i.l.o. effect at each separate binding site, rather than it being a passive phenomenon linked only to the probabilities of torsional flexion.

(*b*) *Receptor-induced ligand orientation* (*r.i.l.o.*) *effects*

Analogy of the concept

The simplest way to visualize the principles of receptor-induced ligand orientation effects is to consider an analogy with two docking space vehicles. In this situation, sensing devices on the docking module collect information about the attitude, velocity and position of the target by interaction of a detecting probe with an electromagnetic field radiated by the latter vehicle. This information is transmitted to rocket thrusters which modulate the attitude and momentum of the approaching vehicle. Hence, there is a sensing and feedback system providing information about the relative position of the docking partners and a driving force which engineers the two modules into the docking position. In the calculation of drug–receptor interaction, where each component is charged, both the sensing and motive force fields are combined in the single interaction of the two electrostatic vector fields. Hence, in this situation the docking manoeuvre is completely automatic, since feedback information and attractive forces are mediated through the same agency. It is not our intention to confine the flight path to an unique line; a number of curved trajectories may be equally possible, depending on the initial velocity and direction of motion of the ligand. In this simplified anionic site model for the receptor fields, it was not possible to calculate oblique trajectories because the base pairs, which would provide a pertubation of the electrostatic fields into the receptor cavity, were omitted.

Kinetic and entropy factors

Molecules in collision *in vacuo* exchange rotational energy at very large distances (reactive cross sections sometimes exceeding 1000 Å^2 for polar species), which indicates that the interaction potential is orientation dependent and that the interaction imposes a torque on the colliding members (Levine & Bernstein 1974). It follows that the long range part of the intermolecular potential cannot be a central potential only, but must contain an anisotropic component. This long range interaction is the electrostatic potential resulting from interactions of the ion–ion, ion–dipole and dipole–dipole type. The average rotational energy of molecules at ambient temperature is approximately 1.25 kJ mol^{-1} for each degree of rotational freedom. Thus the configurational energies calculated here (12–293 kJ mol^{-1}) provide sufficient torque to perturb significantly the thermal distribution of rotational populations illustrated in the Boltzmann probability plots. Theoretical investigations suggest that orienting forces are important in determining the kinetics of reaction (Karplus & Raff 1964; Raff & Karplus 1966;

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Parr *et al.* 1973). These predictions have been substantiated by studies with oriented molecular beams of CH_3I and Rb; the centre of mass differential reactive scattering cross-section ratio was found to be five for favourable and unfavourable orientations (Beuhler & Bernstein 1969). This measurement led to a value for the steric factor and hence to a correct evaluation of the probability of reaction for non-oriented beams.

The importance of orientation effects for a reaction can be estimated from first principles by the methods of molecular reaction dynamics. To do this, one would have to calculate the reaction hypersurface, solve the equations of motion in the potential field and generate a large number of trajectories. Computations of this type would permit a complete description of the rotational and translational pathways of the attacking drug molecule and provide realistic mechanisms of complex formation by including, among others, ping-pong and orbital motions. At present, this is impracticable for large molecules, and, consequently, small regions of the hypersurface have been investigated. The calculations described here examine the position of orientational local minima for two defined points that have been chosen to lie on a path likely to lead to intercalative docking. The results clearly reveal that the approaching ligand experiences a torque which restricts the molecular orientation in response to the variation in receptor field between these two points. Furthermore, they indicate that the preferred orientations adopted by related ligands depend on their electronic properties.

In the absence of sufficient information to perform trajectory calculations, the likely contribution of these orienting forces to the reaction kinetics may be estimated by recourse to transition–state theory. Even with sufficient activation energy, stereospecific interactions between large molecules often proceed at a rate far slower than expected. This is because the pre-exponential term of the Arrhenius rate constant, $k_r = Ae^{-E_0/RT}$, contains a steric factor related to the probability of the reactants having the correct orientations for association to occur. This collision–orientation phenomenon may be expressed in terms of fundamental constants, temperature and canonical partition functions, Q, by the equation

$$k_r = \frac{kT}{h}\frac{Q^*}{Q_A Q_B}\, e^{-E_0/RT},$$

where the subscripts A and B refer to the reactants and the superscript * to the transition–state complex. Evaluation of the canonical partition functions as the molecular partition functions for translation, f_t, rotation, f_r and vibration, f_v, for two nonlinear molecules interacting to form a nonlinear complex yields an expression for the steric factor $(Q^*/Q_A Q_B)$ of $(f_v/f_r)^5$. Substitution of typical values for f_v and f_r produces a steric factor of 10^{-5} (Frost & Pearson 1961). R.i.l.o. computations can be of value in analysis of the importance of the steric factor, since inspection of the probability distribution plots reveals the extent to which the drug is constrained to adopt a suitable orientation along a reactive pathway. For example, a 56.4 kJ mol^{-1} barrier to roll rotations for ethidium results in two orientations suitable for docking, each of which spreads over a rotation angle of about 36° and has a peak probability of approximately 0.1. If only one of these configurations were to lead to a reactive collision and similar restrictions applied to yawing and pitching motions over the interaction hypersurface near the receptor, the rotational partition function, f_r, would be smaller by a factor of 0.1^3. Thus, the steric factor would be increased by 10^3, with a corresponding enhancement in the association rate constant, assuming that the receptor is relatively immobile.

The steric factor can be related to the entropy of activation for the reaction. This is defined

as the difference in entropy between the transition-state complex and the reactants from which it is formed. The major contributions to this entropy result from the loss of translational and overall rotational entropy when the reacting molecules associate. Entropy dissipation before the approaching molecule enters into the transition state provides the driving force for the increased rate, which can be attributed to favourable molecular alignment from r.i.l.o. effects. It is as though the receptor potential field has provided an 'entropy sink' which has been paid for at the expense of the long range interaction energy. In so doing, the association reaction now proceeds at approximately the diffusion rate for oppositely charged ions since only one, or a few, orientations of the drug are presented to the receptor. Therefore, r.i.l.o. mediated reactions tend to maximize the rate of information transfer, since they satisfy the dual criteria of speed and optimized stereochemical configurations in the association of two biologically important molecules. Similar entropy considerations have been discussed by Jencks (1975) for substrate binding and enzyme catalysis. It should be emphasized that the basic energy terms contributing to r.i.l.o. effects, for example, electrostatic, induction and dispersion energies at large intermolecular separations and delocalization, and electron distribution effects at shorter distances, would also apply to the interaction of a charged receptor with a neutral, but polar, ligand. This fact is demonstrated by the r.i.l.o. calculations for carboxyphenylethidium, which is electrically neutral and very polar.

Flexible molecules and potential uses

The concepts of r.i.l.o. effects have been developed with specific reference to the 'lock and key' model for drug–receptor interaction. These same considerations can be extended to the 'zipper-type' mechanism associated with highly flexible ligands. For polypeptides, the propagation reaction is accompanied by energy and entropy changes associated with bond rotations about the backbone N—C^{α} and C^{α}—C′ single bonds as well as around the side chain C^{α}—C^{β} bond. The latter are generally associated with low energy barriers of about 8–12 kJ mol^{-1}, whereas the conformational energy maps for backbone rotations frequently show large areas with energy barriers up to 25 kJ mol^{-1} above the global minimum (Pullman 1977). The entropy of freezing a freely rotating bond into a rigid conformation is the same for all bonds, being equivalent to 7 entropy units (Page & Jencks 1971). Taken together, these entropy and energy barriers may considerably slow the rotations required to complete the binding of individual segments of the ligand. The energy required to overcome these barriers and associated entropy changes would be derivable in part from interactions of the r.i.l.o. type.

R.i.l.o. effects may have some practical use in the study of drug–receptor interaction. First, drug molecules could be modified to select the best orientation in their flight towards the receptor by use of the electrostatic rudder concept. Secondly, they could be used to alter the position of the drug in the receptor complex to enhance the specificity of binding. For example, the difference in the minimum energy position during yaw rotations of ethidium and its carboxyphenyl derivative is 30°, and an orientation energy difference of 33 kJ mol^{-1} through that angle for carboxyphenylethidium. If the stacking energy of interaction between the nucleic acid base pairs and the phenanthridinium ring is less than 33 kJ mol^{-1}, then one might expect to find a different alignment of the drug in the complex. This idea could be tested by cocrystallization of dC-dG with carboxyphenylethidium. Replacement of the carboxyl group rudder by the extremely polar carbonyl moiety could be advantageous. The free energy

of interaction of the latter derivative with the receptor would not be lowered because of the unfavourable interaction of the full negative charge of the acidic group. It may be possible, by systematic examination of r.i.l.o. effects for different moieties, together with electrostatic field pictures, to develop a set of empirical principles to aid in the prediction of docking orientations.

(*c*) *Energy partition in the docking manoeuvre*

The origin of the r.i.l.o. effect is the spatial dependence of the energy partitioning between the two interacting molecular systems. Partition of the interaction energy can conceptually be split further into intermolecular and intramolecular contributions, although these are interdependent. For ethidium or carboxyphenylethidium interacting with a double anionic charge, two contributions to the total energy predominate: electrostatic and nuclear attractions between the drug and receptor over large distances, and delocalization energy. Intramolecular changes, however, can be attributed to modified electron distributions and to the energy levels of the molecular orbitals. Careful examination of the phenanthridinium ring of ethidium and carboxyphenylethidium can illuminate these intramolecular changes. The electron distribution of the hetero-aromatic ring is markedly influenced by an external anionic field, with the most pronounced effect occurring on the sp^2–hybridized amino nitrogen atom; even at 12 Å the charge on this atom is altered by 25 m*e* for both molecules. Orbital energy levels are very sensitive to the presence of an anionic field, the frontier orbitals being affected at a separation of up to 100 Å from the source of the anionic charge.

The effect of orientation on alteration of the partition of the interaction energy of ethidium and a double anionic site can be attributed to the properties of the large heteroaromatic ring; the electrons here appear to be fairly mobile. This finding may have an important consequence for studies of the interaction with nucleic acid base pairs in the stacked drug–receptor complex; a purine·pyrimidine base pair may be thought of as another large heteroaromatic ring. If the interaction energy is dependent on the whole of the three heteroaromatic ring systems, it would seem unwise to attempt a stacking energy calculation based on piecemeal addition of the separate interaction energies with each nucleic acid base.

The primary objective of this paper was to determine whether the drug molecule approaches the receptor in a random orientation for collision or whether the drug is aligned for an effective docking. We have established that there are considerable energy barriers to rotation of the ligand round the three Eulerian angles of roll, yaw and pitch in the field of the receptor. These barriers restrict the freedom for independent movement. The energetic constraints to rotation are of quantum mechanical origin, the main components being (i) electrostatic and (ii) induced electron delocalization in the drug molecule. Mapping the molecular electrostatic potentials generated by the two docking partners is a very helpful method of visualizing the electrostatic effects. Modifications of the molecular structure of the drug were found to alter the alignment angles in the docking manoeuvre. Therefore, in the docking of a drug molecule towards its receptor, the relative motion is controlled by a finely tuned intermolecular guidance mechanism, the essential elements of which can be resolved by quantum mechanical analysis.

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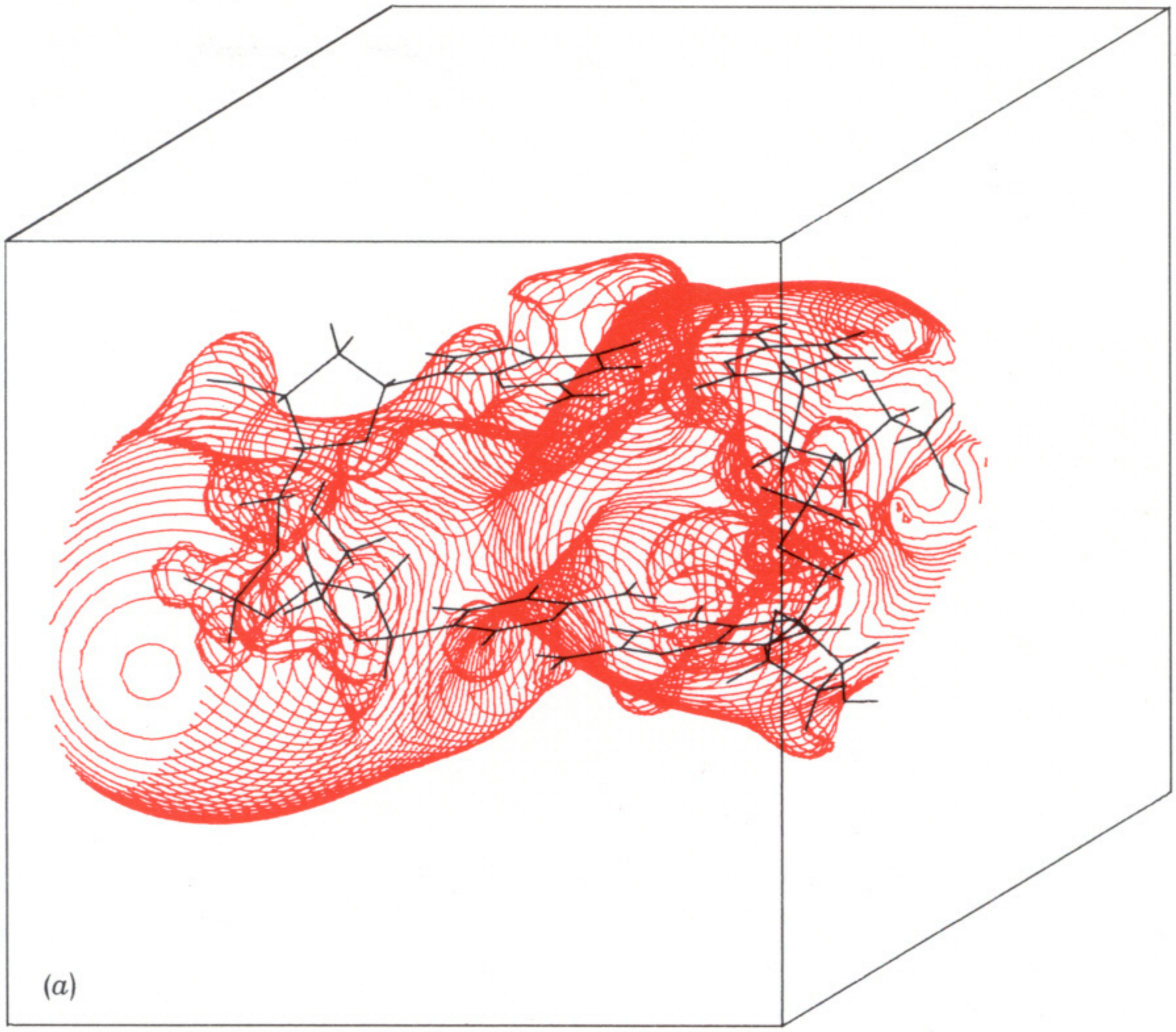


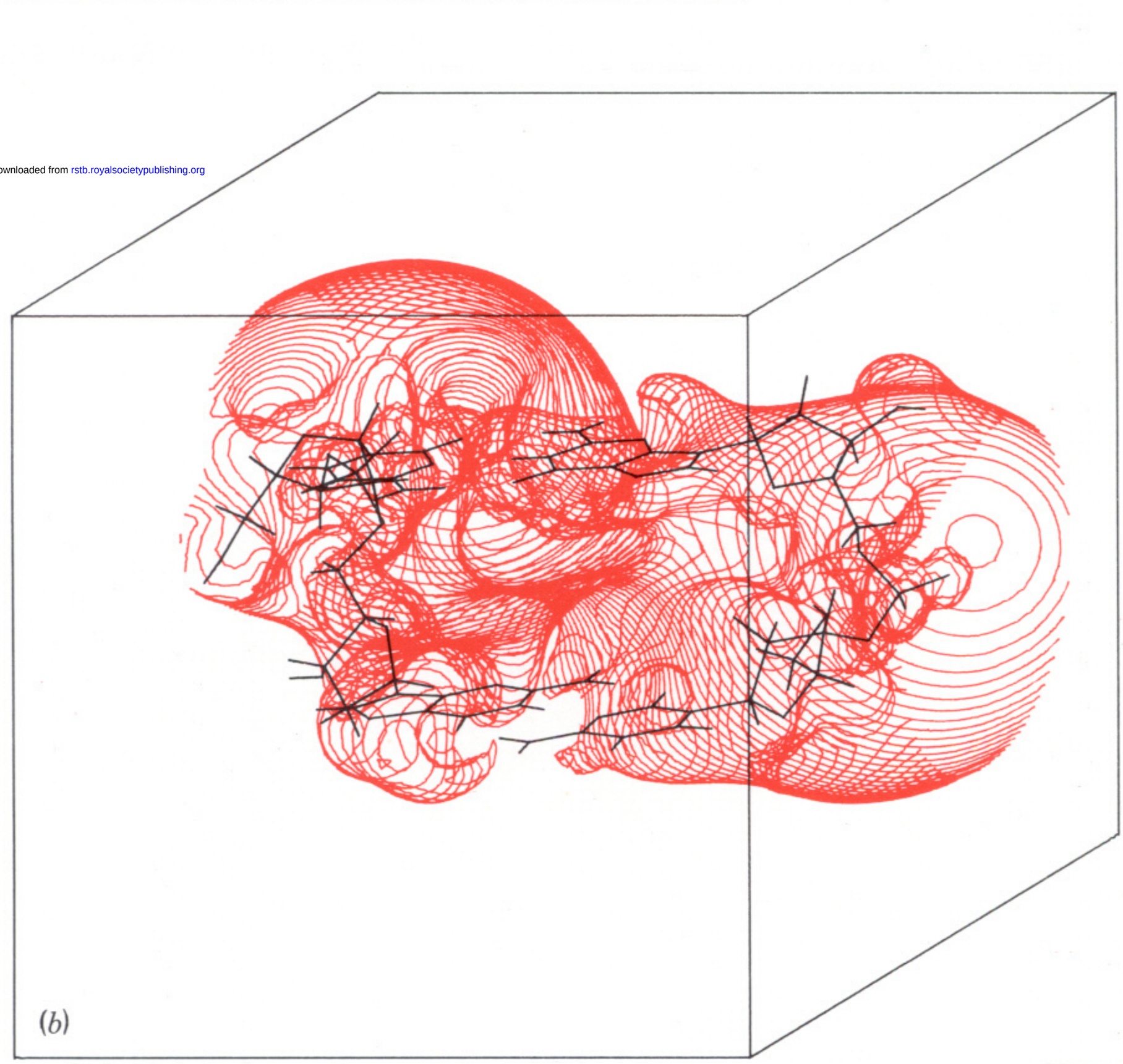
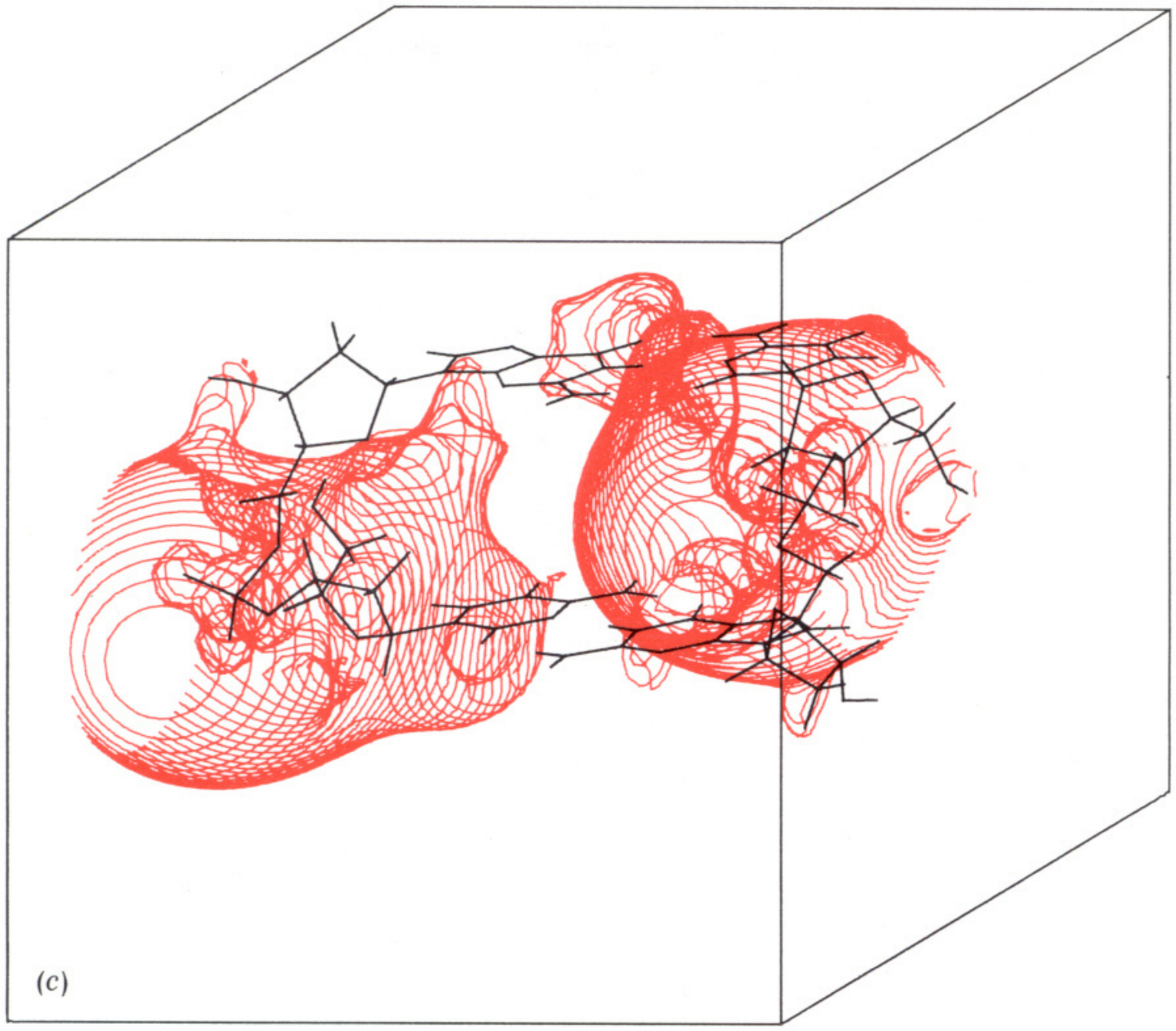


FIGURE 4. Three-dimensional molecular electrostatic potential surfaces for the receptor (dC–dG) · (dC–dG). Bonds are shown by the skeleton drawings superimposed on the field contours. Potential surface –334 kJ mol^{-1} with (*a*) the narrow groove and (*b*) the wide groove towards the front face.



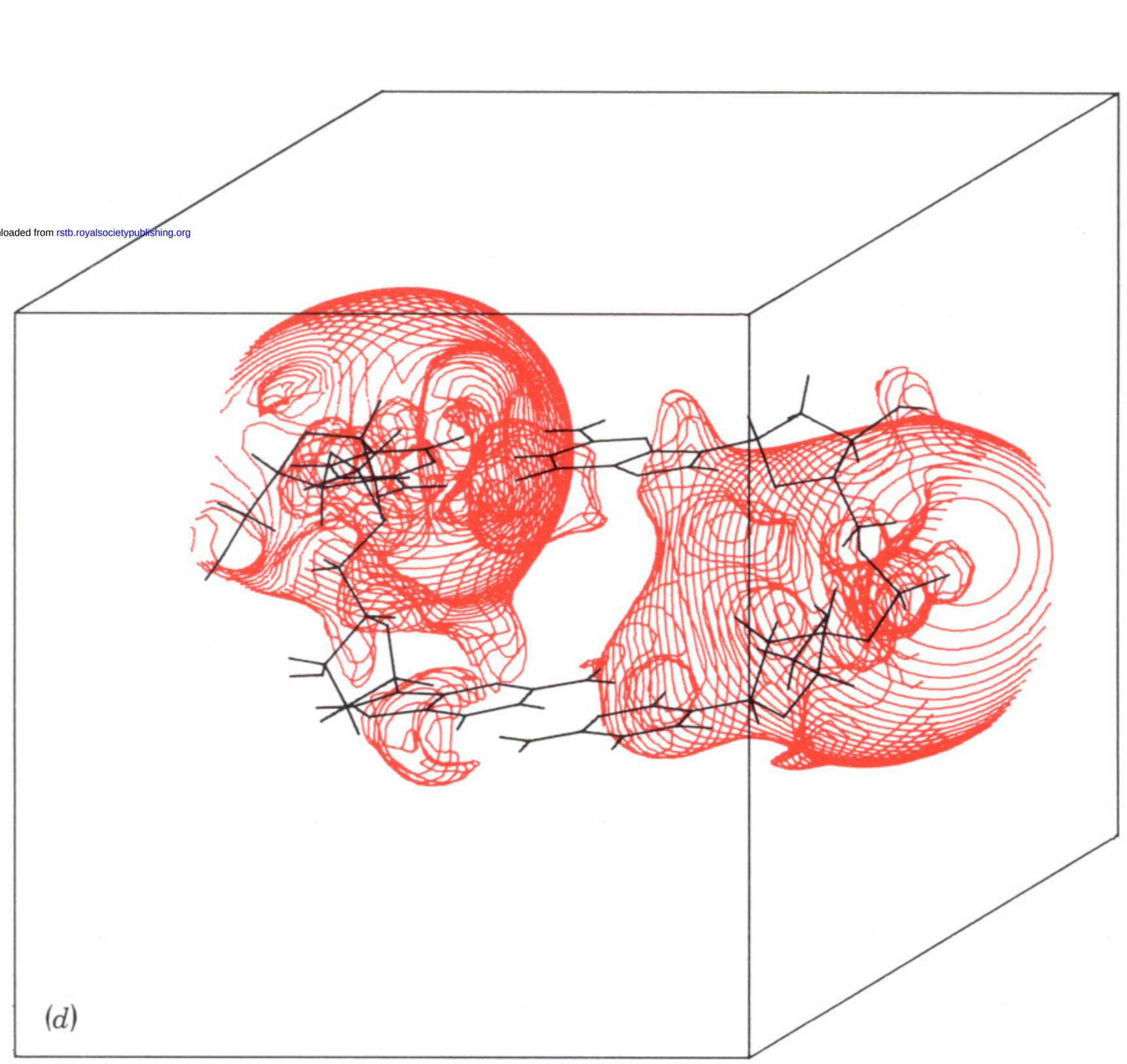


FIGURE 4 (continued). Potential surface –418 kJ mol^{-1} with (*c*) the narrow groove and (*d*) the wide groove towards the front face.

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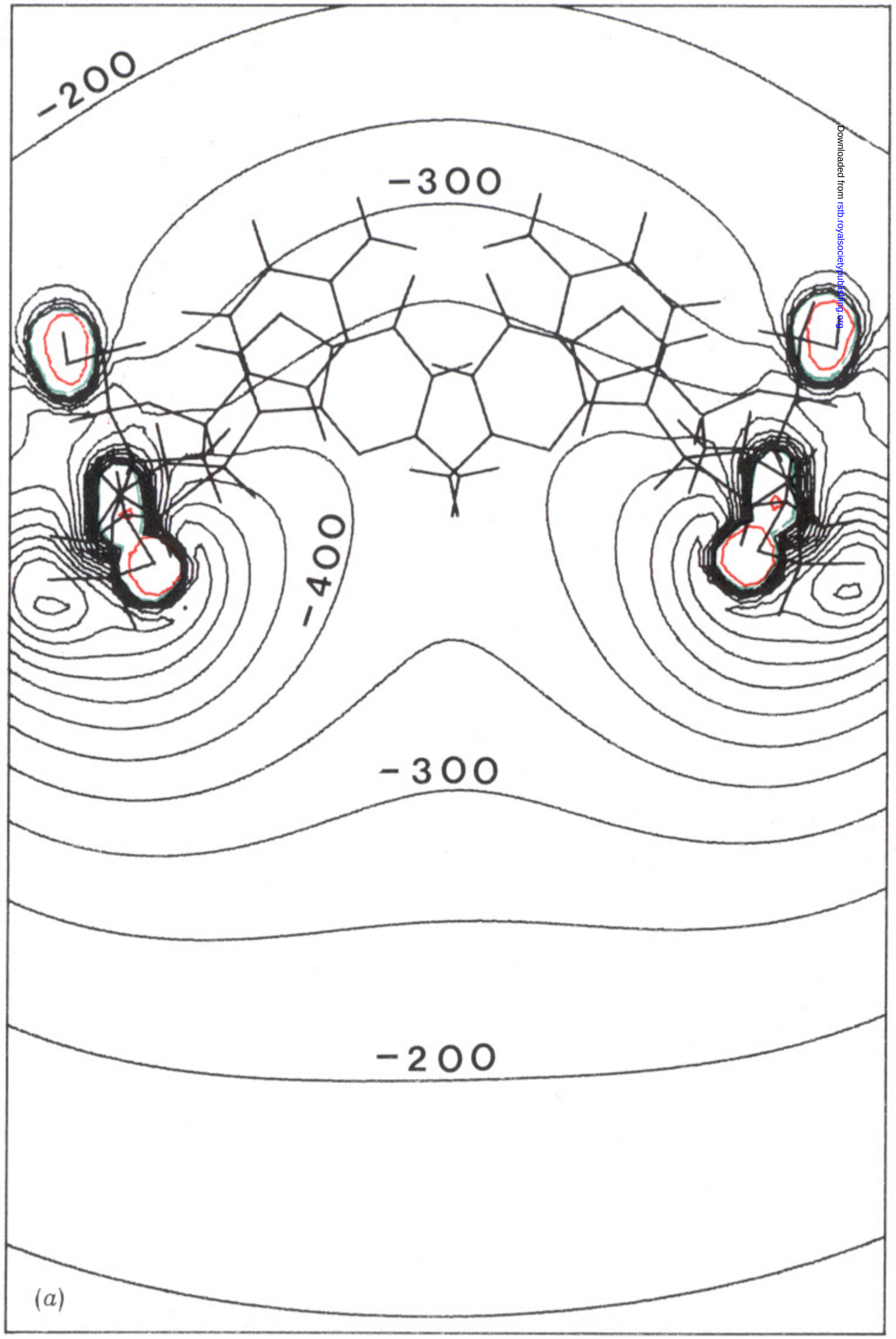

FIGURE 5. Electrostatic contour maps through the intercalation plane of the receptor. Narrow groove facing the lower part of the frame. Red contour +418 kJ mol^{-1}, green contour 0 kJ mol^{-1}, black contours at −50 kJ mol^{-1} intervals. (*a*) Isolated receptor. (*b*) Ethidium at 12Å from the intercalated position.

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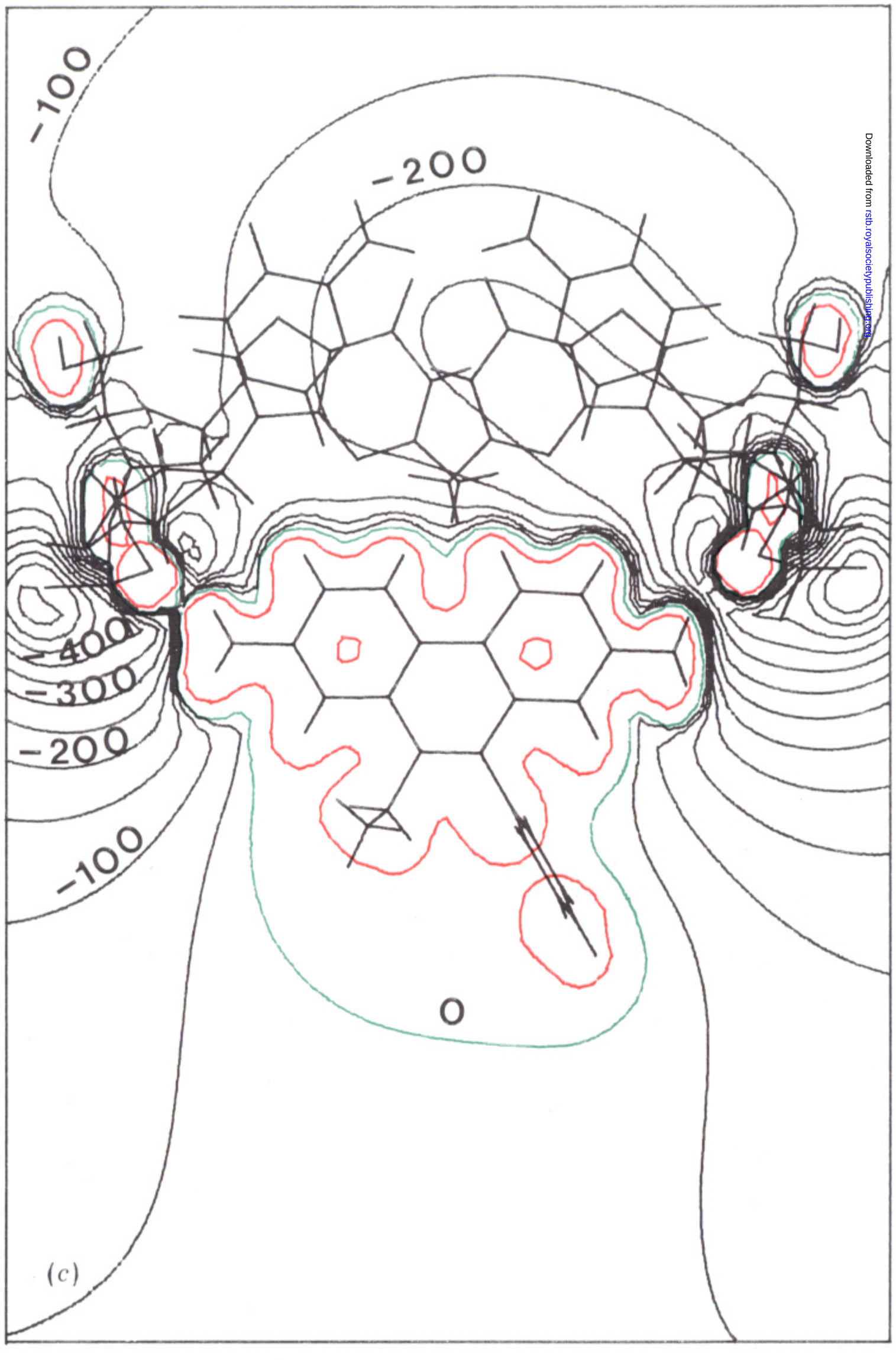

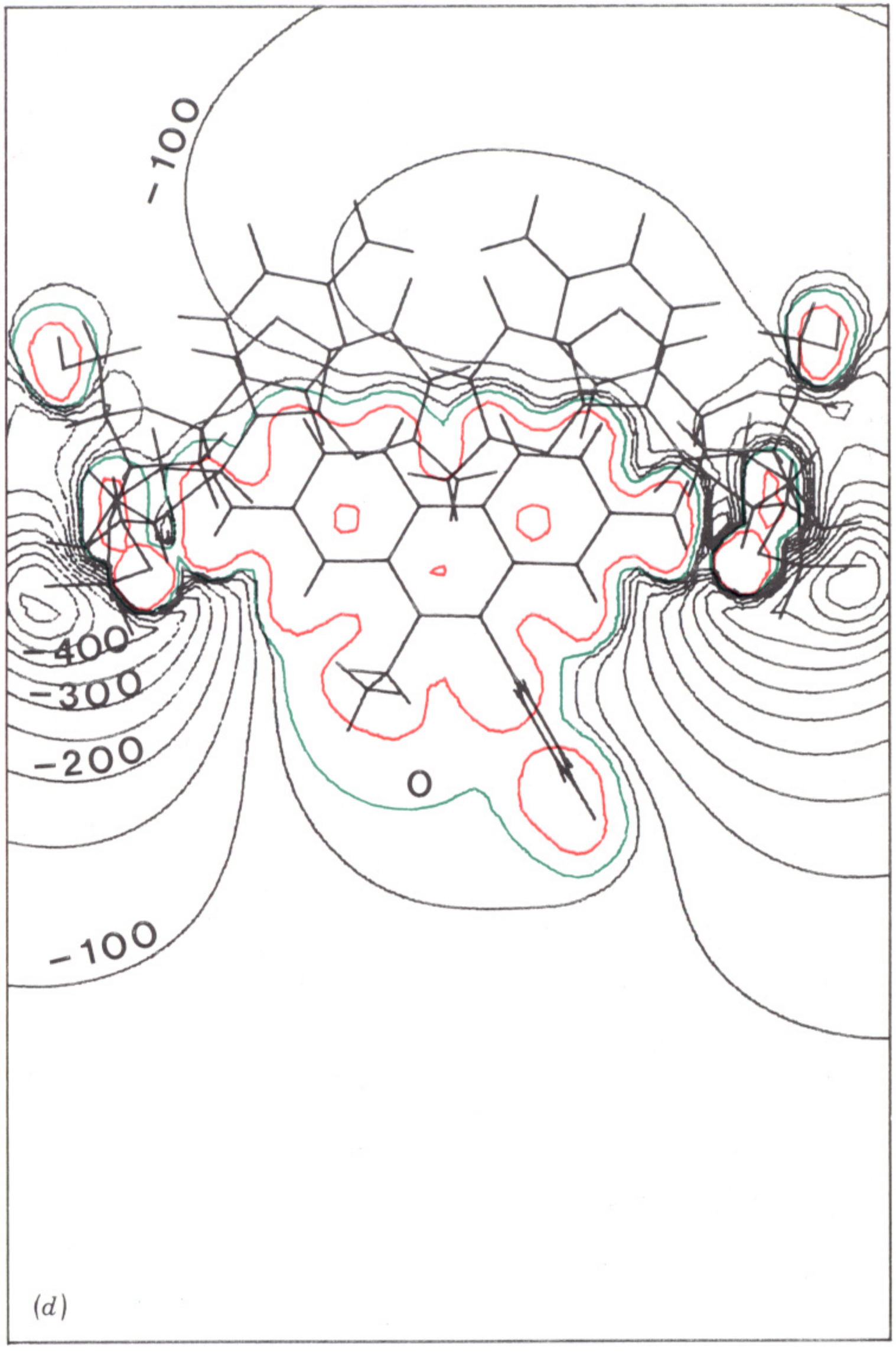

FIGURE 5 (continued). (*c*) Ethidium at 6Å from the intercalated position. (*d*) Ethidium at 3Å from the intercalated position.

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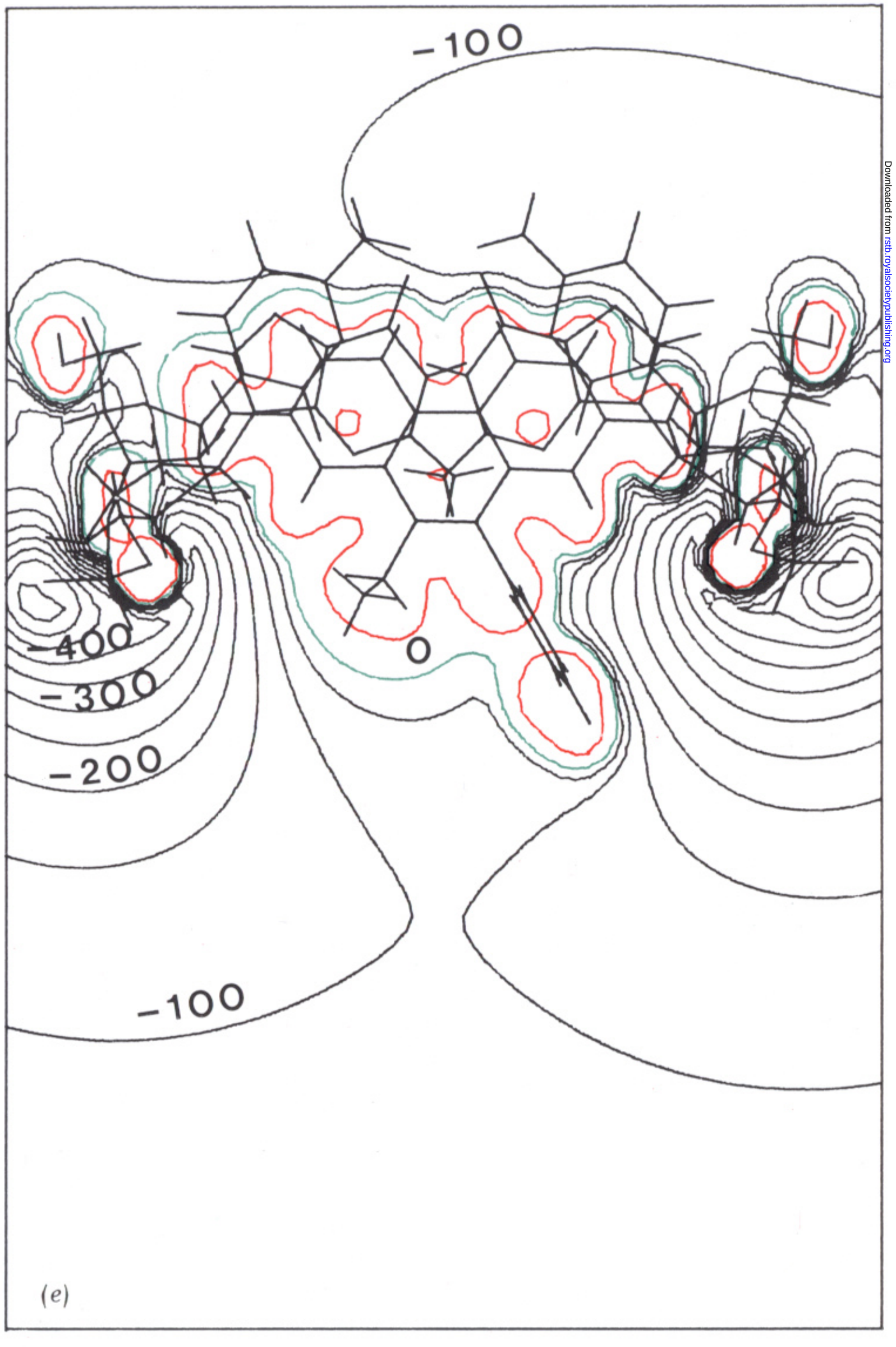

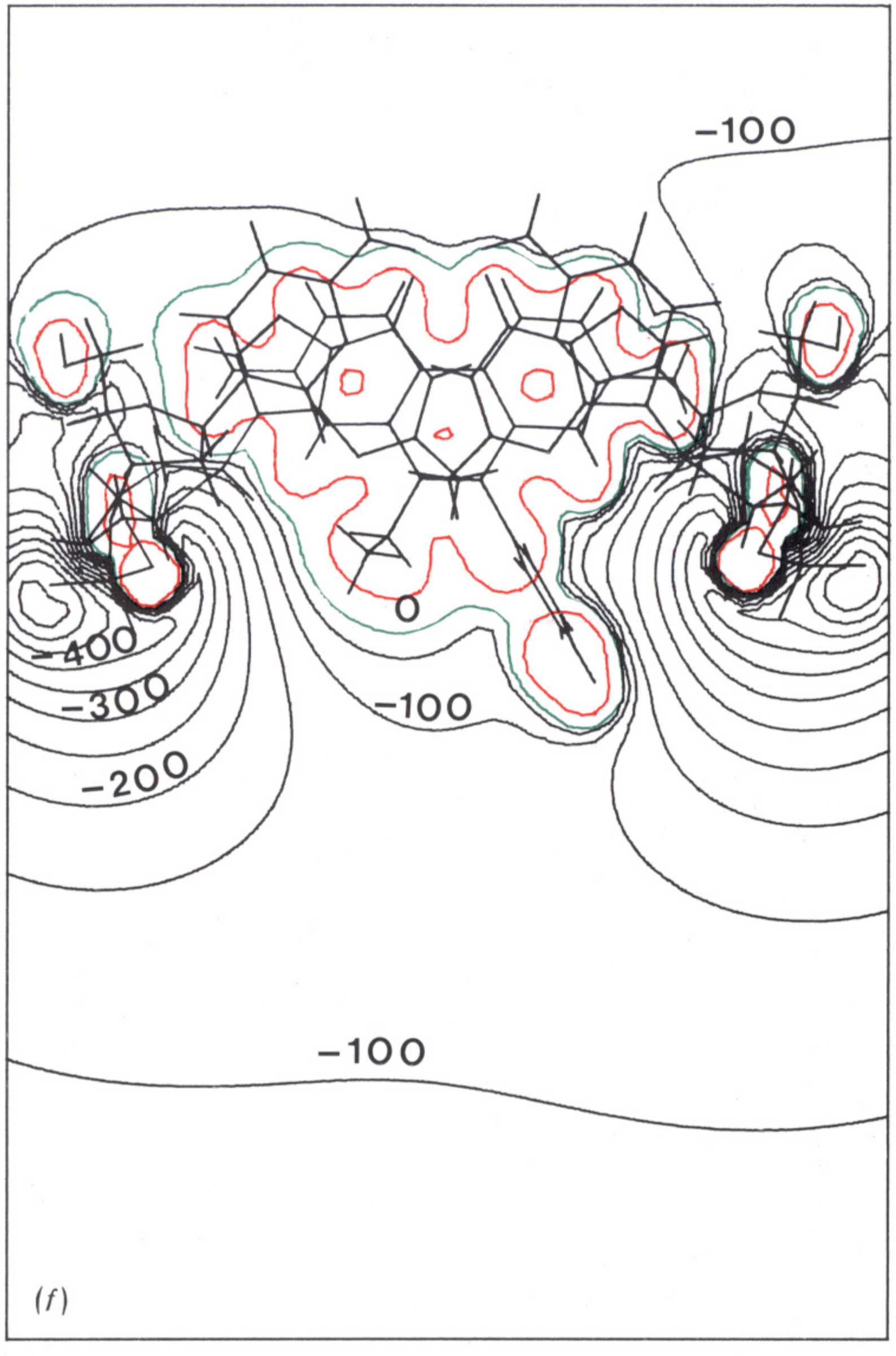

FIGURE 5 (continued). (*e*) Ethidium at 1 Å from the intercalated position. (*f*) Ethidium fully intercalated.

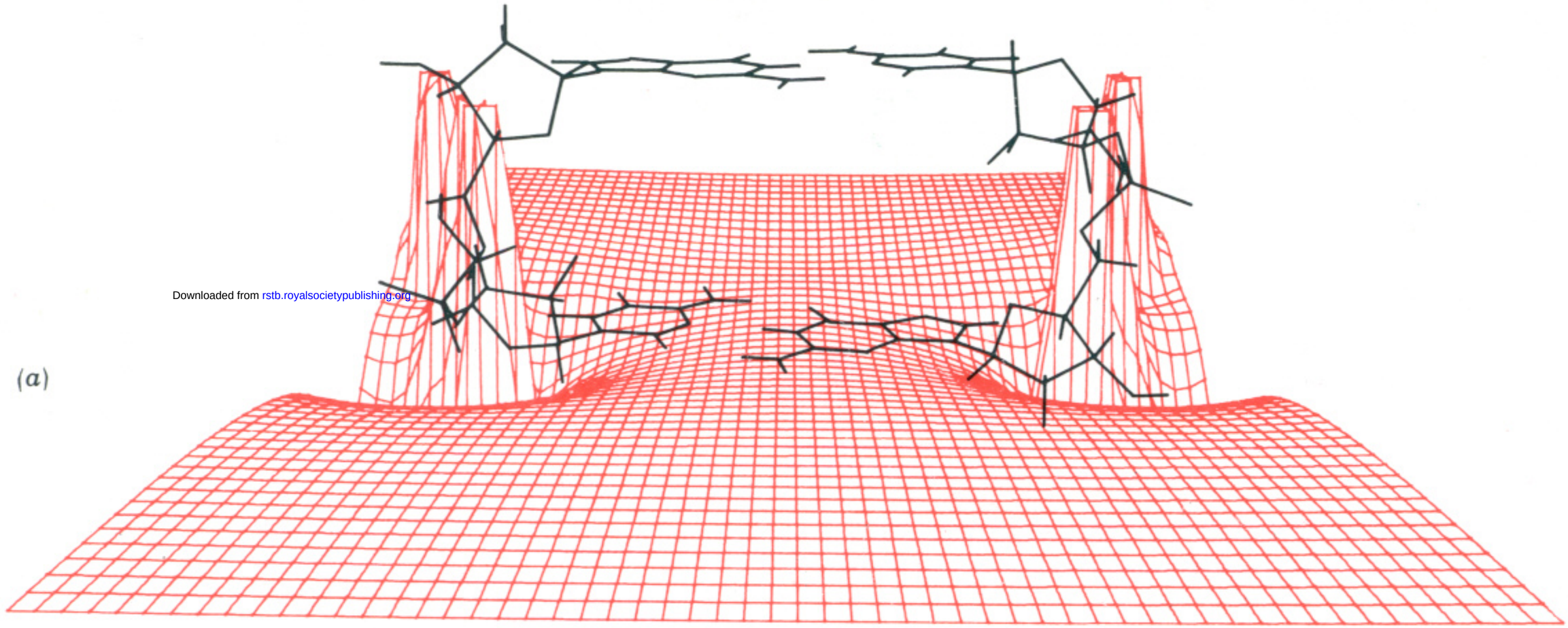

FIGURE 6. Hidden line suppression profiles of the electrostatic potential in the intercalation plane. (*a*) Data taken from figure 5*a* and viewed through the receptor from the narrow groove side. (*b*) Data taken from figure 5*b* and viewed through the receptor from the wide groove towards the docking ethidium molecule.

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