The use of the electrostatic potential at the molecular surface in recognition interactions: Dibenzo-*p*-dioxins and related systems

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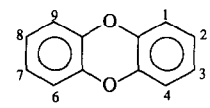
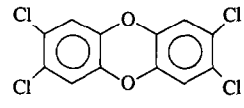
An ab initio self-consistent-field molecular orbital approach was used to compute the electrostatic potentials of dibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), two analogues of the latter, and two isomeric benzoflavones on a three-dimensional molecular surface corresponding to the contour of constant electronic density equal to 0.002 electrons/bohr^3. The results are discussed in relation to the biological activities of the respective molecules. It is shown that the electrostatic potential graphically depicted on the molecular surface is well suited for the study of recognition interactions, such as are believed to be involved in the initial receptor-mediated step leading to toxicity in the dibenzo-p-dioxins. The surface potential has the advantage of clearly showing steric features that may play a role in understanding the recognition process being investigated.

Keywords: electrostatic potential, molecular surface, dibenzo-p-dioxins, benzoflavones, recognition interactions

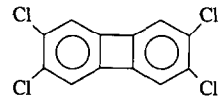
The electrostatic potential $V(\mathbf{r})$ that the nuclei and electrons of a molecule create in the surrounding space has been shown to be a very useful analytical tool in the study of molecular reactivity,[1-5] including biological recognition interactions.[5-12] In earlier studies, we have presented the electrostatic potentials of dibenzo-*p*-dioxin (**I**), and a variety of halogenated dibenzo-*p*-dioxins, including the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, **II**), and several structural analogues of TCDD (such as **III** and **IV**). We have identified patterns in these potentials that are associated with high degrees of biological activity,[13-17] including toxicity, aromatic hydrocarbon hydroxylase (AHH) induction, and receptor binding. In addition, we have studied the electrostatic potentials of the two isomers, 5,6-benzoflavone (**V**) and 7,8-benzoflavone (**VI**)[16]; these are also



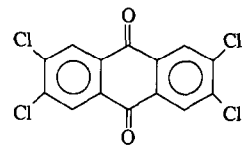
I



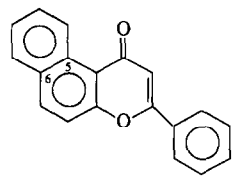
II



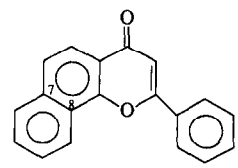
III



IV



V



VI

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inducers of AHH activity, but are inhibitors of the subsequent metabolic oxidation carried out by this enzyme system.[18]

In light of the evidence that the toxic effects produced by the dibenzo-*p*-dioxins and related systems are receptor-mediated,[19-21] our earlier work focused on the initial recognition process involved in this receptor interaction. We computed $V(\mathbf{r})$ at a distance of 1.75 Å above the molecular plane, which corresponds to the approximate van der Waals radius of the largest atom in this group of molecules (chlorine).[22] This is the potential that a receptor would encounter during a recognition process. In systems with central oxygens, $V(\mathbf{r})$ was also computed in perpendicular planes through the oxygens to study the effects of structure and substitution on their characteristic negative potentials.

We have recently developed a procedure for viewing and analyzing the electrostatic potential on a three-dimensional surface, designed to encompass nearly all of the electronic charge of the molecule.[23] This approach has been shown to be effective for successfully interpreting and predicting nucleophilic processes,[23] which previously have been much less straightforward to interpret (by means of electrostatic potentials) than electrophilic ones.[3,4]

In the present work, our aim is to demonstrate that the electrostatic potential plotted on a well-defined molecular surface is well suited for the study and analysis of receptor interactions and complements our earlier two dimensional approach. Accordingly, the surface potentials of **I–VI** will be presented and discussed in the light of their respective biological activities and in relation to our previous work in this area.

METHODS AND PROCEDURE

The molecular electrostatic potential at any point $\mathbf{r}$ is expressed rigorously by

$$V(\mathbf{r}) = \sum_A \frac{Z_A}{|\mathbf{R}_A - \mathbf{r}|} - \int \frac{\rho(\mathbf{r}')\, d\mathbf{r}'}{|\mathbf{r}' - \mathbf{r}|} \qquad (1)$$

where Z_A is the charge on nucleus A, located at $\mathbf{R}_A$, and $\rho(\mathbf{r})$ is the electronic density function of the molecule, which we obtain by an ab initio self-consistent-field molecular orbital approach. The first term on the right side of equation 1 represents the contribution of the nuclei, which is positive; the second term brings in the effect of the electrons, and is negative. An important feature of $V(\mathbf{r})$ is that it is a real physical property, which can be determined experimentally by diffraction techniques as well as computationally.[4]

In this work, $V(\mathbf{r})$ has been computed and graphically displayed on a characteristic three-dimensional surface, corresponding in all instances to the contour of constant electronic density equal to 0.002 electrons/bohr^3. It has been shown, for a group of diatomic molecules and for methane, that this contour in three dimensions encompasses at least 95% of the electronic charge and gives physically reasonable molecular dimensions.[24-26] This surface is defined in terms of a molecular property, the electronic density function $\rho(\mathbf{r})$, and thereby reflects features such as bond formation and lone pairs that are unique to a molecule. This is in contrast to earlier approaches in which surfaces have been defined in terms of sets of intersecting spheres centered on the nuclei of the molecule.[24,27-37]

Because of the size of molecules **I–VI**, crystallographic geometries have been used whenever possible as starting points for our computations. The structure of TCDD (**II**) was taken from a crystallographic study[38] and used as a model for **I**, with appropriate modifications. Chlorines were replaced by hydrogens in **I**, with CCH angles set to 120° and all C—H bond lengths taken to be 1.08 Å. For **III**, a geometry was approximated, with C—C bond distances in the aromatic rings set to 1.38 Å, and the two C—C bonds joining these rings set to 1.40 Å. The C—Cl and C—H bond distances in **III** were set to 1.73 and 1.02 Å, respectively; the CCX angles (where X is H or Cl) were taken to be 120°. The structure of **IV** was taken from a crystal structure of anthraquinone,[39] with the C—Cl bond distances and C—C—Cl angles set to 1.73 Å and 120°, respectively. For **V** and **VI**, structures were obtained from crystallographic data.[40]

Using these geometries, we have computed STO-5G wave functions for **I–VI** with the GAUSSIAN 86 system of programs.[41] We have found this basis set to be satisfactory for properties related to the electronic charge distribution, as is $V(\mathbf{r})$. Furthermore, extensive investigations have shown that ab initio SCF approaches, such as are used in this study, produce generally reliable representations of the electrostatic potential.[1,2,42,43] These wave functions were then used to calculate surface electrostatic potentials. The latter can, of course, be shown at whatever degree of resolution is desired. For the molecules **I–VI**, we have chosen to indicate three ranges of $V(\mathbf{r})$, by means of different colors.

RESULTS AND DISCUSSION

The calculated electrostatic potentials on the molecular surfaces of the parent inactive dibenzo-*p*-dioxin, **I**, and the highly toxic TCDD, **II**, are shown in Color Plates 1 and 2. In Color Plate 1, negative potentials extend over the outer two rings, becoming stronger near the central oxygens. The lateral regions (near positions 2,3 and 7,8) are positive, as is the surface above the central dioxin ring. The negative potentials above the outer rings can be interpreted as being due to the aromatic π electrons.

The surface $V(\mathbf{r})$ of TCDD (Color Plate 2) is in extreme contrast to that of **I**. Negative lateral regions associated with the chlorines are separated by a large positive $V(\mathbf{r})$ region. The negative potentials above the outer rings of **I** are completely eliminated, as was seen also in our earlier two-dimensional $V(\mathbf{r})$ plots.[13] In addition, the oxygen negative potentials in **II** are greatly diminished relative to **I**, and appear on the sides of the surface, largely masked from view. These various features reflect the strong electron-attracting inductive power of the chlorine substituents.

2,3,6,7-Tetrachlorobiphenylene (**III**) is a non-oxygen-containing analogue of TCDD, very similar to **II** in toxicity, AHH induction, and receptor binding. Its surface electrostatic potential, presented in Color Plate 3, is also strikingly similar to that of TCDD (Color Plate 2); negative lateral regions are separated by an extensive area of positive potential. This points out, as we have suggested earlier,[16] that oxygens in the central portion of the molecule are not required for high activity.

It is seen in Color Plate 4 that the surface $V(\mathbf{r})$ of the relatively inactive 2,3,6,7-tetrachloroanthraquinone (**IV**) has a pattern similar to those of **II** and **III** (Color Plates 2 and 3). However, the negative potentials associated with the central oxygens in **IV** are farther apart from each other and more accessible than those of TCDD (Color Plate 2). This difference between the potentials of **II** and **IV**, which is steric in nature, is clearly brought out by Color Plates 2 and 4. These results are consistent with our earlier speculation that the biological inactivity of **IV** may be due in part to the carbonyl oxygen negative regions being in a position to inhibit an attractive interaction between **IV** and the receptor binding site, whatever that may be.[16]

Our present results for **I**–**IV** support our earlier observations regarding factors that are linked to high biological activities in the dibenzo-*p*-dioxins and similar systems.[13,16] Negative regions above all or most of the lateral positions of the molecule, separated by an area of positive potential, appear to be necessary for high activity; oxygens in the central portion of the molecule are not. Our results for the highly toxic **II** and the relatively inactive **IV** suggest that in cases with central oxygens, a high level of biological activity requires their characteristic negative potentials to be small, relatively weak, and close to the center of the molecule. These features are graphically depicted by the electrostatic potentials on the molecular surfaces of the highly active **II** and **III** (Color Plates 2 and 3). In the case of **IV**, the steric factor that may be responsible for its low activity (more widely separated and accessible negative oxygen potentials) is also clearly shown by its surface potential (Color Plate 4).

5,6-Benzoflavone (**V**) and 7,8-benzoflavone (**VI**) are structural isomers differing in the position of a fused benzene ring. **V** is essentially planar, whereas in **VI** the linked phenyl ring is rotated slightly out of the plane of the remainder of the molecule.[40] The calculated electrostatic potentials on the molecular surfaces of **V** and **VI** (Color Plates 5 and 6, respectively) show negative regions that can be attributed to the carbonyl oxygens, the ring oxygens, and the π electrons of the aromatic rings. However, the overall $V(\mathbf{r})$ patterns are qualitatively different for the two molecules. In **VI**, the strongest negative regions are associated with the two oxygens, while in **V** they are with the carbonyl oxygen and the nearby 5,6-benzo ring, and overlap due to their proximity; the ring oxygen is less negative. The nonplanarity of **VI** is clearly indicated by its surface potential. (Note the tilting of the linked phenyl ring in Color Plate 6.)

The surface electrostatic potentials of **V** and **VI** present a different picture of the carbonyl oxygen regions than did our earlier two-dimensional $V(\mathbf{r})$ plots.[16] The latter showed the carbonyl region of **VI** to be significantly more negative than that of **V**. This probably reflects the difficulty in choosing appropriate planes in which to compare $V(\mathbf{r})$ for planar and nonplanar systems, such as **V** and **VI**. The computation of $V(\mathbf{r})$ on a well-defined molecular surface is clearly advantageous in this respect; the necessity of selecting planes is eliminated, thus removing any ambiguity associated with this choice.

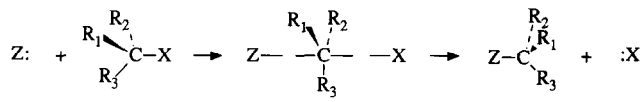
The surface potentials of **V** and **VI** can help to explain their differing biological activities. **V** is known to be a stronger inducer of AHH activity than **VI** and has a higher binding affinity to the cytosolic Ah receptor.[18] Perhaps the negative regions of the carbonyl and ring oxygens in **VI** somewhat interfere with binding to the receptor, resulting in a diminished tendency to induce AHH activity (relative to **V**). The nonplanarity of **VI** may be a further factor affecting receptor binding.

Once the AHH activity has been induced, **V** and **VI** actually inhibit the metabolic oxidation carried out by this enzyme system. Both the oxidation and the inhibition are believed to occur at the cytochrome P-450 enzyme site.[18] Our surface potential analysis supports earlier speculation as to why **VI** is a more effective inhibitor than **V**.[44] The unhindered situation of the negative carbonyl oxygen region of **VI** may allow it to displace a ligand at the P-450 enzyme site (one of them is known to be weakly bound), thereby interfering with any subsequent oxidation–reduction reactions. This type of inhibition conceivably could not occur with **V**, with its shielded carbonyl oxygen. It is therefore more likely that **V** acts as a competitive inhibitor, mimicking the substrate and blocking the enzyme active site.

To further demonstrate the effectiveness of surface electrostatic potentials in accounting for steric effects, such as appear to be of considerable importance in understanding the biological activities of the systems discussed thus far, we present the electrostatic potentials on the molecular surfaces of methyl chloride (**VII**) and *tert*-butyl chloride (**VIII**).

$H_3C{-}Cl$ **VII** $\qquad$ $(H_3C)_3C{-}Cl$ **VIII**

In aliphatic systems, the S_N2 displacement of a group X by a nucleophile Z: proceeds through the transition state **IX** and thus involves inversion of configuration:



IX

For a given Z, the reactivity of the chlorine in this substitution diminishes in the order

$$H_3C{-}Cl > (H_3C)H_2C{-}Cl > (H_3C)_2HC{-}Cl > (H_3C)_3C{-}Cl$$

a trend that has been attributed to the steric hindrance due to the methyl groups.[45]

This is reflected in the surface potentials of **VII** and **VIII**, shown in Color Plates 7 and 8, respectively. (STO-3G optimized structures were used to compute $V(\mathbf{r})$ at the 3-21G level.) The yellow areas, with $V(\mathbf{r}) > 20$ kcal/mol, are indicative of regions highly susceptible to nucleophilic attack. The large yellow area that can be seen in Color Plate 7 for $H_3C{-}Cl$ is almost completely absent in Color Plate 8*a*, which shows the corresponding view for $(H_3C)_3C{-}Cl$. There is still a yellow region, but it is buried inside the cone formed by the $C{-}CH_3$ bonds (Color Plate 8*b*).

SUMMARY

We have computed and presented the electrostatic potentials on the molecular surfaces of dibenzo-*p*-dioxin, 2,3,7,8-

tetrachlorodibenzo-*p*-dioxin, two analogues of the latter, and two isomeric benzoflavones. We have discussed these in the light of the molecules' respective biological activities. Our results are consistent with those obtained earlier with two-dimensional plots of $V(\mathbf{r})$ for these and a variety of related systems. However, the use of surface potentials eliminates all uncertainty related to choosing the most appropriate plane in which to compute the potential; all molecules are now treated in a consistent manner. This can be rather an important point for systems with significant asymmetry, such as the benzoflavones. The surface electrostatic potentials clearly reveal steric features that may affect the recognition process being investigated. Their effectiveness in accounting for steric factors is further demonstrated by the comparison of H_3C—Cl and $(H_3C)_3C$—Cl. Our results show that the electrostatic potential graphically depicted on an appropriate three-dimensional surface is well suited for the study of recognition interactions, such as are believed to be involved in the initial receptor-mediated step leading to the biological activities of the dibenzo-*p*-dioxins and related systems.

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REFERENCES

1. Scrocco, E., and Tomasi, J. In *Topics in Current Chemistry,* Springer-Verlag, Berlin, 1973, No. 42, p. 95
2. Scrocco, E., and Tomasi, J. *Adv. Quantum Chem.* 1978, **11**, 115
3. Politzer, P., and Daiker, K. C. In *The Force Concept in Chemistry,* B. M. Deb, Ed. Van Nostrand Reinhold, New York, 1981, p. 294
4. Politzer, P., and Truhlar, D. G., Eds. *Chemical Applications of Atomic and Molecular Electrostatic Potentials,* Plenum, New York, 1981
5. Politzer, P., Laurence, P. R., and Jayasuriya, K. *Environ. Health Perspect.* 1985, **61**, 191 (Special Issue on Structure-Activity Correlation in Mechanism Studies and Procedure Toxicology)
6. Loew, G. H., and Berkowitz, D. S. *J. Med. Chem.* 1975, **18**, 656
7. Petrongolo, C., and Tomasi, J. *Int. J. Quantum Chem., Quantum Biol. Symp.* 1975, **2**, 181
8. Osman, R., Weinstein, H., and Topiol, S. *Ann. N. Y. Acad. Sci.* 1981, **367**, 356
9. Weinstein, H., Osman, R., Green, J. P., and Topiol, S. In *Chemical Applications of Atomic and Molecular Electrostatic Potentials,* P. Politzer and D. Truhlar, Eds. Plenum, New York, 1981, p. 309
10. Martin, M., Sauz, F., Campillo, M., Pando, L., Perez, J., and Turvar, J. *Int. J. Quantum Chem.* 1983, **23**, 1627, 1643
11. Thomson, C., and Brandt, R. *Int. J. Quantum Chem., Quantum Biol. Symp.* 1983, **10**, 357
12. Politzer, P., and Murray, J. S. In *Theoretical Biochemistry and Molecular Biophysics: A Comprehensive Survey,* D. L. Beveridge and R. Lavery, Eds. Adenine, Schenectady, NY, in press
13. Murray, J. S., Zilles, B. A., Jayasuriya, K., and Politzer, P. *J. Am. Chem. Soc.* 1986, **108**, 915
14. Murray, J. S., and Politzer, P. *Theor. Chim. Acta* 1987, **72**, 507
15. Politzer, P. *Toxicol. Lett.* 1988, **43**, 257
16. Murray, J. S., Evans, P., and Politzer, P. *Int. J. Quantum Chem.,* 1990, **37,** 271
17. Murray, J. S., Grice, M. E., Politzer, P., and Rabinowitz, J. R. *J. Comput. Chem.,* 1990, **11,** 112
18. Rossi, M., Cantrell, J. S., Farber, A. J., Dyott, T., Carrell, H. L., and Glusker, J. P. *Cancer Res.* 1980, **40**, 2774
19. Poland, A., and Knutson, J. C. *Annu. Rev. Pharmacol. Toxicol.* 1982, **22**, 517
20. Poland, A., Greenlee, W. F., and Kende, A. S. *Ann. N. Y. Acad. Sci.* 1979, **330**, 214
21. Goldstein, J. A. In *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzo-p-dioxins and Related Products,* Elsevier, New York, 1980, pp. 151–190
22. Bondi, A. *J. Phys. Chem.* 1964, **68**, 441
23. Sjoberg, P., and Politzer, P. *J. Phys. Chem.,* in press
24. Kahn, S. D., Parr, C. F., and Hehre, W. J. *Int. J. Quantum Chem., Quantum Chem. Symp.* 1988, **22**, 575
25. Bader, R. F. W., Henneker, W. H., and Cade, P. E. *J. Chem. Phys.* 1967, **46**, 3341
26. Bader, R. F. W., and Preston, H. J. T. *Theor. Chim. Acta* 1970, **17**, 384
27. Pullman, B., Perahia, D., and Cauchy, D. *Nucleic Acids Res.* 1979, **6**, 3821
28. Weiner, P. K., Langridge, R., Blaney, J. M., Schaeffer R., and Kollman, P. A., *Proc. Natl. Acad. Sci. U.S.A.* 1982, **79**, 709
29. Connolly, M. L. *Science* 1983, **221**, 709
30. Bash, P. A., Pattabiraman, N., Huang, C., Ferrin, T. E., and Langridge, R. *Science* 1983, **222**, 1325
31. Francl, M. M., Hout, R. F., Jr., and Hehre, W. J. *J. Am. Chem. Soc.* 1984, **106**, 563
32. Quarendon, P., Naylor, C. B., and Richards, W. G. J. *J. Mol. Graphics* 1984, **2**, 4
33. Connolly, M. L. *J. Am. Chem. Soc.* 1985, **107**, 1118
34. Ritchie, J. P. *J. Am. Chem. Soc.* 1985, **107**, 1829
35. Burridge, J. M., Quarendon, P., Reynolds, C. A., and Goodford, P. J. *J. Mol. Graphics* 1987, **5**, 165
36. Bader, R. F. W., Carroll, M. T., Cheeseman, J. R., and Chang, C. J. *J. Am. Chem. Soc.* 1987, **109**, 7068
37. Arteca, G. A., Jammal, V. B., Mezey, P. J., Yadav, J. S., Hermsmeier, M. A., and Gund, T. M. *J. Mol. Graphics* 1988, **6**, 45
38. Boer, F. B., Remoortere, F. P., North, P. P., and Newman, M. S. *Acta Crystallogr. Sect. B.* 1972, **28**, 1023
39. Prakash, A. *Acta Crystallogr.* 1967, **22**, 439
40. Rossi, M., Cantrell, J. S., Farber, A. J., Dyott, T.,

Carroll, H. L., and Glusker, J. P. *Cancer Res.* 1980, **40**, 2774
41. Frisch, M. J., Binkley, J. S., Schlegel, H. B., Raghavachari, K., Melius, C. F., Martin, R. L., Stewart, J. J. P., Bobrowicz, F. W., Rohlfing, C. M., Kahn, L. R., DeFrees, D. J., Seeger, R., Whiteside, R. A., Fox, D. J., Fleuder, E. M., and Pople, J. A. *GAUSSIAN 86,* Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh, PA, 1984
42. Ghio, C., and Tomasi, J. *Theor. Chim. Acta* 1973, **30**, 151
43. Almlof, J., and Stogard, A. *Chem. Phys. Lett.* 1974, **29**, 418
44. Ullrich, V., and Schnabel, K. H. *Drug Metab. Dispos.* 1973, **1**, 176
45. Kemp, D. S., and Villaccio, F. *Organic Chemistry.* Worth, New York, 1980