BIOCHEMICAL JOURNAL LETTERS

Biological activity of polychlorinated biphenyls related to conformational structure

The biological properties of polychlorinated biphenyls (PCBs) vary considerably and are remarkably dependent on the number and positions of halogen atoms in the molecular structure (Goldstein, 1980). Because *ortho* substitution plays a dominant role in determining the pivot bond or phenyl-ring twist angle, it would be desirable to be able to calculate accurately conformational energy difference in order to classify the various PCB structures in correlations with biological activity.

Sassa *et al.* (1986) used MNDO (modified neglect of diatomic overlap), a semi-empirical geometry optimization method which has been parameterized with experimental data (Dewar & Thiel, 1977*a*,*b*) to study PCB twist angles. However, when this method is applied to related systems with an sp^2–sp^2 formal single bond (Choudhury & Scheiner, 1984), an energy-minimized structure near 90° (double bond plane twist or dihedral angle) is found. This result is in disagreement with experimental results for non-*ortho*-substituted halogenated biphenyls [X-ray (McKinney & Singh, 1980) or gaseous phase (Almenningen *et al.*, 1985) structures] but in agreement with the results of Sassa *et al.* (1986) investigating the conformational energy of a number of PCBs with various degrees of *ortho*-substitution by the same method. In addition we have recently compared the minimum energy structures of several related stilbene structures with X-ray structures using molecular mechanics (MM2p), MNDO and *ab initio* (STO-3G) calculations (Darden *et al.*, 1986). In all cases, the MNDO method gave large average absolute errors (25–27° deviations) in estimating torsional angles found in the X-ray structures. The smallest error (3–12° deviations) was found using MM2p with similar results for *ab initio* calculations when applied to a symmetrical structure.

With regard to non-*ortho*-substituted biphenyls, we (McKinney *et al.*, 1983*a*) have found using *ab initio* calculations (STO-3G) that both biphenyl and three non-*ortho* chlorine-substituted biphenyls had minimum-energy structures at approx. 42°. Similar results were reported for biphenyl by others using the STO-3G basis set (Häfelinger & Regelmann, 1985; $\phi_{min.} = 38.63°$) and split valence basis set (Almlof, 1974; $\phi_{min.} = 32°$). We have also found (Singh *et al.*, 1987) that molecular mechanics (MM2p) gives results for a non-*ortho*-substituted halogenated biphenyl in close agreement with X-ray (near 35°). A twist angle of 90° as suggested by MNDO calculations is clearly not the correct minimum energy structure for all PCBs. Thus while the MNDO method may be of some qualitative value in classifying *ortho*-substituted PCB structures, we would caution against its use if any sort of quantitative structure–activity relationship is being sought for all PCBs.

Using the *ab initio* and molecular mechanics methods to assign PCBs to stereochemical classes, we have developed a theoretical model for the interactions of PCBs with cytosol receptors (dioxin or Ah receptor) interpreted in terms of a linear free energy relationship (McKinney *et al.*, 1983*a*,*b*, 1984, 1985). The essential molecular parameters in this model are the PCB polarizability and the receptor–PCB separation distance which depends on steric factors. The advantage of this model is that it incorporates both the stereoelectronic and energetic effects of chlorine substitution on the binding interaction. In view of the work of Sassa *et al.* (1986) on uroporphyrinogen decarboxylase activity, we used porphine as our receptor binding model because of its obvious similarity to the PCB structures in planar geometrical extent. We commented on the possible relationship of a binding interaction of this type to the potential of PCBs to produce porphyria (McKinney *et al.*, 1983*b*). The important aspect of our work was not necessarily to describe the microenvironment of the binding site but rather to develop a theoretical model (QSAR) based on molecular parameters of PCBs that correlated reasonably with the experimental binding results. A graphic representation of this model, which was based on molecular mechanics minimization of the PCB–porphine interactions (McKinney *et al.*, 1985) was also presented. This work suggested that the *ortho*-effect is important in determining the PCB–receptor separation distance and that the dispersion energy gain from a coplanar alignment may assist in overcoming the small rotational barrier to planarity in non-*ortho*-substituted PCB. We believe that if differences in PCB solubilities, desolvation energies and nonspecific binding under experimental bioassay conditions are properly accounted for, this model can be useful in deriving quantitative structure–activity relationships for other kinds of biological activity which may have predictive value.

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Chloro-substituent sites and probability of co-planarity in polychlorinated biphenyls in determining uroporphyrin formation in cultured liver cells

We agree with McKinney & Pedersen [1] in that MNDO, a semi-empirical molecular orbital method, may not give the perfect solution for calculating the conformational energy of polychlorinated biphenyls (PCBs). It is clear that appropriate precautions are necessary for the use of a semi-empirical method.

McKinney & Pedersen [1] raised a question about the use of MNDO (modified neglect of diatonic overlap) for calculating the conformational energy of PCBs. The two main points of their criticism are (i) calculated results by MNDO in an sp^2–sp^2 system do not agree with experimental data obtained by X-ray crystallography or gaseous phase, and (ii) although MNDO may be applicable to *ortho*-substituted congeners, it is not suitable for calculating the conformational energy of non-*ortho*-substituted molecules. They also compared the minimum energy structures of several related stilbenes with X-ray structures using (i) molecular mechanics (MM2p), (ii) MNDO and (iii) *ab initio* (STO-3G) and report that the MNDO gave large average absolute errors in estimating torsional angles found in the X-ray structures. Based on these findings, McKinney & Pedersen [1] caution against the use of MNDO for quantitative structure–activity relationship studies on PCBs.

Perhaps it is necessary to discuss why we chose MNDO for the calculation of the conformational energy of PCBs to seek for a possible structure–activity relationship of PCBs and uroporphyrin formation in PCB-treated cultured liver cells [2]. It is reasonable to expect, using MNDO, that a certain trend can be resolved for the structure–activity relationship of PCBs if the method is applied to a group of several related congeners. For example, Kaminsky *et al.* [3] made molecular orbital calculations on dichlorobiphenyls by using MNDO for the structure–activity relationship of their metabolism by purified rat liver cytochrome *P*-450 species and were able to make certain predictions of the necessary structure for metabolism. For these reasons, we chose MNDO for the molecular orbital calculation of PCBs for the study of the structure–activity relationships of PCBs on uroporphyrin formation.

Our results demonstrated that, based on the conformational energy, which is calculated as a function of the dihedral angle between the two phenyl rings, biphenyl congeners can be classified into four groups with different conformations. The conformation of active PCBs was relatively flexible, whereas inactive species had a rigidly angulated conformation. Furthermore, the calculated probability of the conformation distribution for each congener indicated that the probability of co-planarity was higher for active biphenyls than for inactive congeners. This is one of our two important conclusions which distinguish the active PCBs from the inactive congeners. The other is that at least two halogens must be present at lateral adjacent sites in both phenyl rings. Based on these findings, we suggested that both the chloro-substituent sites and the probability of co-planarity are important in determining the inhibitory effect of PCBs on uroporphyrinogen decarboxylase activity.

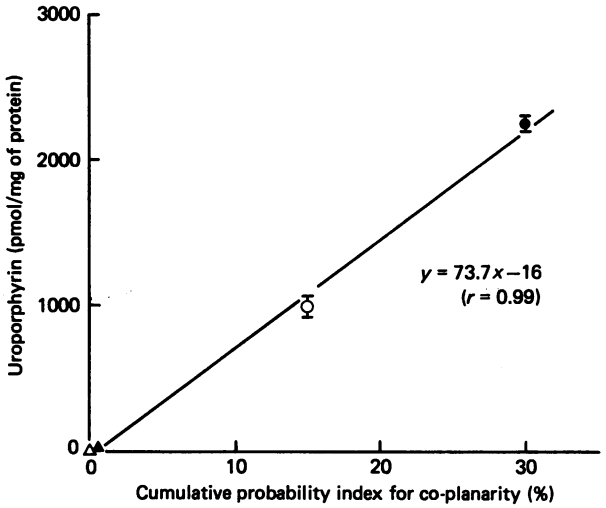

Fig. 1. Relationship between cumulative probability index for congener co-planarity and uroporphyrin formation

●, 3,4,3′,4′-tetrachlorobiphenyl; ○, 2,4,3′,4′-tetrachlorobiphenyl; ▲, 2,3,2′,3′-tetrachlorobiphenyl; △, 2,6,2′,6′-tetrachlorobiphenyl.

The model proposed by McKinney & Pedersen [1] stresses the importance of the polarizability of PCBs and the receptor–PCB separation distance. On the other hand, we introduced the concept that the cumulative probability index for congener co-planarity is critical in determining uroporphyrin formation in cultured chick embryo hepatocytes with PCBs. Our data clearly showed that the cumulative probability index for co-planarity for the four representative PCB groups was closely correlated ($r = 0.99$) with uroporphyrin accumulating activity (Fig. 1). It was not our intention to conclude that the use of MNDO yields an absolutely exact figure; instead we demonstrated that the data obtained by using MNDO were in excellent agreement with our experimental data, i.e. uroporphyrin formation in PCB-treated liver cultures.