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Lipid Binding and Mode of Action of Compounds of the Dichlorodiphenyltrichloroethane Type: A Proton Magnetic Resonance Study

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SUMMARY

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An interaction between compounds of the dichlorodiphenyltrichloroethane type (*p,p'*-DDT, *o,p*-DDT, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, methoxychlor, dicofol, and DDA) and lecithin has been studied by nuclear magnetic resonance spectroscopy. The PMR spectrum of lecithin in CCl_4 showed chemical shift changes upon the addition of DDT type compounds, particularly in the resonance peak of the choline protons. Conversely, when lecithin was added to a dilute solution of these compounds, it produced chemical shift changes in the benzylic proton as well as in the ring protons. These changes have been explained on the basis of complex formation involving an acidic proton of the DDT type compounds and the phosphate of the lecithin. The chemical shift changes were used to calculate the equilibrium constant as well as the chemical shift changes of the complex. Correlations can be shown between the chemical shift changes and the toxicity of the DDT type compounds.

INTRODUCTION

The toxicity of dichlorodiphenyltrichloroethane and related compounds is due to an effect on the central nervous system, specifically to an effect on axonic transmission. Changes in the action potential have been related to impaired ion (K^+, Na^+) efflux, and the results suggested that DDT[1] modifies membrane permeability (1, 2). In view of its very low solubility in water, some interaction with the membrane lipoprotein complex seems likely. DDT will complex with components of cockroach nerve, and Matsumura and O'Brien have postulated from spectral studies (3–5) that a charge transfer complex is involved. Also, Hilton and O'Brien (6) have shown that DDT blocks the action of valinomycin on a lecithin-decane bilayer.

In carbon tetrachloride solution DDT binds to lecithin (7). The most plausible mechanism for such an interaction is the association of the negatively charged phosphate oxygen with the benzylic proton, whose acidic character is due to the elec-

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[1] The abbreviations used for DDT and similar compounds are defined by the structures shown in Table 1.

tronic effects of the chlorine substituents in the DDT molecule. Whether such an interaction is of biological significance will be answered ultimately by comprehensive studies of the interaction of DDT in systems which more closely approximate biological conditions. Meanwhile some circumstantial evidence can be derived from the study of a series of DDT-like compounds, relating their biological activity to the characteristics of their interaction with lecithin.

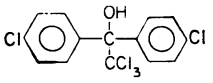
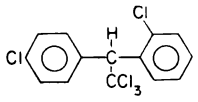
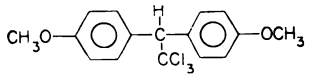
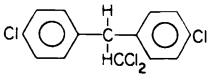
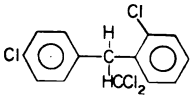
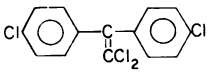
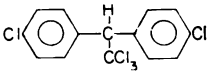
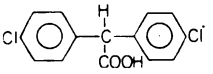
In this paper we describe the binding of eight DDT type compounds to a phospholipid, lecithin. The proton magnetic resonance technique has been used as a tool, and the results are discussed in terms of the toxicity of these compounds.

MATERIALS AND METHODS

Lecithin (β,γ-dipalmitoyl-DL-phosphatidylcholine) was obtained from Sigma Chemical Company and was used without further

TABLE 1

Interaction of DDT-like compounds with dipalmitoyllecthin: equilibrium constants and changes in chemical shift for the pure complexes

Compound	Structure	Proton studies	Equilibrium constant, K	Chemical shift change, $\Delta\delta_c$
			molal^{-1}	*Hz*
1,1,1-Trichloroethanes *p,p'*-DDT		Benzylic Ring	0.598 ± 0.16[a] 0.716 ± 0.30	58.3 ± 9.2[a] 18.8 ± 5.6
o,p-DDT		Changes in chemical shift too small for calculation		
Methoxychlor		Benzylic Ring	2.52 ± 0.28 2.31 ± 0.46	55.44 ± 1.42 4.31 ± 1.89
1,1-Dichloroethanes *p,p'*-DDD		C-1 Ring	2.21 ± 0.04 3.69 ± 0.04	104.0 ± 3.7 12.6 ± 0.4
o,p-DDD		C-1	1.47 ± 0.08	151.2 ± 12.2
1,1-Dichloroethane DDE		No interaction observed		
1,1,1-Trichloroethanol Dicofol (Kelthane)		Ring	56.4 ± 0.4	5.7 ± 0.2
Acetic acid DDA		Ring	29.2 ± 0.1	4.66 ± 0.10

[a] Error term derived from error terms for slope and intercept of regression equation.

purification. The DDT type compounds used were obtained from City Chemical Corporation and are listed in Table 1. The carbon tetrachloride used was of spectrograde quality. PMR spectra were recorded on a Varian HA 100-MHz spectrometer at 33°, with tetramethylsilane as a lock standard.

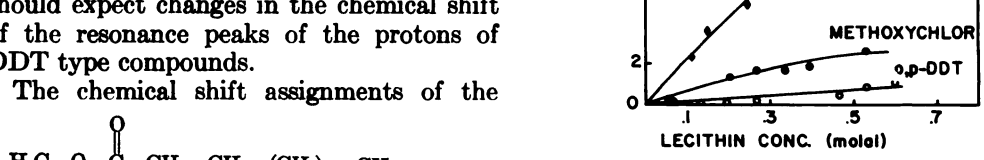
To observe the effect of lecithin on the chemical shift changes of the various DDT type compounds, low concentrations of the latter were used: *p,p'*-DDT and *o,p*-DDT, 1.4×10^{-2} molal; *o,p*-DDD, 1.5×10^{-2} molal; *p,p'*-DDD, 2.2×10^{-2} molal; *p,p'*-DDE, 1.57×10^{-2} molal; *p,p'*-DDA, 1.78×10^{-2} molal; methoxychlor, 1.49×10^{-2} molal; dicofol, 1.4×10^{-2} molal. Under these conditions the chemical shifts of the various proton peaks of the DDT type compounds were not concentration-dependent. However, the concentrations were adequate to provide good PMR spectra.

RESULTS AND DISCUSSION

The assignment of peaks to the PMR spectrum of lecithin in CCl_4 has been discussed earlier (8). The addition of various DDT type compounds to a dilute lecithin solution produced changes in the chemical shift of the peaks due to the choline methyl protons as well as the *N*-methylene protons (I). These changes were similar to those observed for the interaction of DDT with lecithin (7), indicating the involvement of the phosphorylcholine moiety in the interaction. The DDT type compounds containing a slightly acidic proton interact with the negative phosphate oxygen. Changes in the electronic environment of the choline protons would be expected; hence the low-field change in resonance peaks. Conversely, one should expect changes in the chemical shift of the resonance peaks of the protons of DDT type compounds.

The chemical shift assignments of the PMR spectra of the compounds investigated were in agreement with those reported by Keith *et al.* (9). When lecithin was added to dilute solutions of these compounds, low-field changes in chemical shift were observed for the resonance peaks of various protons. The magnitude of the change in chemical shift varied with the compound (7).

The large change (low-field) in chemical shift of the benzylic proton of *p,p'*-DDT produced by increasing concentrations of lecithin is illustrated in Fig. 1a. Methoxychlor and *o,p'*-DDT gave a much smaller



I

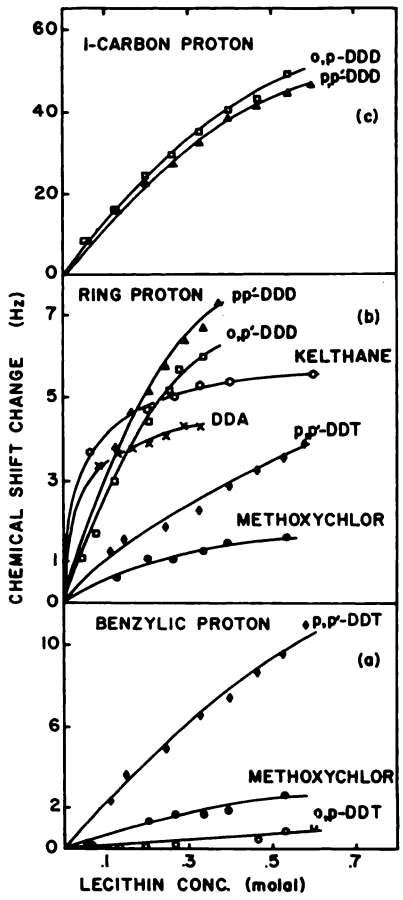


FIG. 1. *Changes in chemical shift of various protons of DDT type compounds with addition of lecithin*

The concentrations of DDT type compounds were: *p,p-* and *o,p*-DDT, 1.4×10^{-2} molal; methoxyclor, 1.49×10^{-2} molal; *p,p'*-DDD, 2.2×10^{-2} molal; *o,p*-DDD, 1.5×10^{-2} molal; *p,p*-DDA, 1.78×10^{-2} molal; dicofol, 1.39×10^{-2} molal.

response. At low concentrations of lecithin (below 0.125 molal) the chemical shift of the benzylic proton of neither DDD isomer was affected, while at higher concentrations the peak due to this proton was masked by the lecithin spectrum. Similar interference precluded monitoring of the benzylic proton of DDA.

For those compounds substituted in the *para,para'* positions it is possible to classify the ring protons into two groups, those *meta* to the benzylic carbon and those in the *ortho* position. Each group has a doublet with further fine splitting (9). In all cases it was the latter group which showed change in chemical shift with increased lecithin concentration (Fig. 1b). The former group was not affected. The ring protons of the *ortho,para* isomers of DDT and DDD gave a more complex spectrum (9). One prominent, sharp peak was obtained along with smaller peaks. Changes in the chemical shift of the larger peak were noted (Fig. 1b). Lecithin did not produce any change in the PMR spectrum of DDE.

The largest changes in chemical shift produced by added lecithin were observed with the proton on carbon 1 of the DDD isomers (Fig. 1c). This proton gave a doublet due to spin-spin splitting (J = 8.5 Hz) with the benzylic proton (9).

Resonance peaks due to the ring protons of dicofol (Kelthane) showed definite line broadening (Fig. 2), which was dependent on the concentration of lecithin, indicating a much slower rate of exchange of dicofol between the free and complexed state (10).

These changes in the chemical shift of various protons of DDT type compounds could result either from self-association of such compounds or from complexation with lecithin. The former could involve simple intermolecular interactions, such as those suggested by Schaefer and Schneider (11) for substituted benzenes, or vertical stacking, as observed in aqueous solutions of purine nucleosides (12). However, in both these instances one should expect concentration dependence in the chemical shift of various protons of DDT type compounds. No such concentration dependence of chemical shift has been observed either in this study or in comparable studies of Ross and

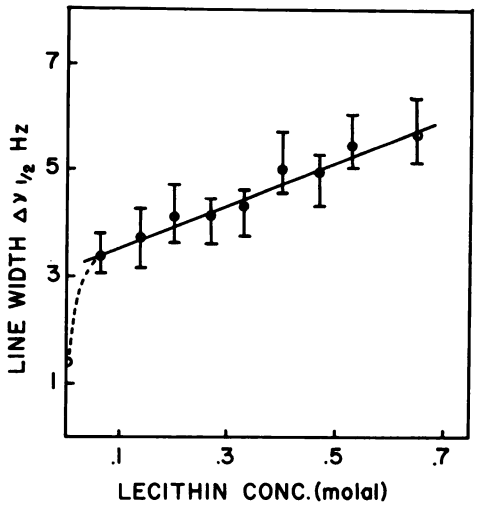


FIG. 2. *Changes in linewidth of ring protons of dicofol (Kelthane) with addition of lecithin*

Biros (13) and Wilson (14). We thus conclude that the observed chemical shift changes in DDT type compounds are due to complex formation with lecithin. Similar conclusions have been drawn from the PMR studies of the interaction of DDT with benzene and related compounds (13, 14). In this case the formation of a charge transfer complex is suggested. More recently Wilson and Wilson (15) have confirmed the involvement of the phosphate moiety in the binding of a phospholipid to DDT.

With dilute solutions of the compounds under investigation, the observed changes in the chemical shift ($\Delta\delta_o$) of the affected proton may be related to the concentration of added lecithin, C, by the expression (16)

$$\frac{1}{\Delta\delta_o} = \frac{1}{K\Delta\delta_c} \cdot \frac{1}{C} + \frac{1}{\Delta\delta_c}$$

With observations of the changes in chemical shift at a series of lecithin concentrations a regression analysis provides values for the equilibrium constant (K) and the change in chemical shift for the pure complex ($\Delta\delta_c$) (Table 1). These two quantities provide contrasting information on the interaction. The over-all tendency for these two molecular species to associate in CCl_4 is given by K, while the degree of involvement of a particular proton is indicated by $\Delta\delta_c$.

It appears that K values for most of the compounds under investigation were of about the same magnitude (0.6–3.0). A hy-

droxyl or carboxyl substitution in the ethane moiety apparently enhances the interaction, as is evident from the high K values of dicofol and DDA. The results also suggest that DDE does not bind to lecithin whereas o,p'-DDT interacts to a very limited extent. In view of the strong dependence of K on the dielectric constant of the medium, its magnitude may change significantly in aqueous solution.

On the formation of a complex, two major processes produce changes in chemical shift: (a) polarization, which induces a distortion of the electronic structure of the bond in question, and (b) an effect due to magnetic anisotropy (17). In the former an inverse relation exists between change in chemical shift and the square of the distance separating the proton from the interacting site. The contribution in chemical shift ($\Delta\delta_a$) due to the anisotropic effect can be expressed as

$$\Delta\delta_a = \frac{\Delta\chi}{3r^3}(1 - 3\cos^2\theta)$$

where $\Delta\chi$ is the difference in the magnetic susceptibility of the anisotropic group parallel and perpendicular to the bond axis, θ is the angle between the latter axis and the radius vector, and r is the magnitude of the radius vector drawn from the proton to the center of anisotropy. Changes in the ring current upon complex formation could also affect chemical shift (18). The converse relation between change in chemical shift and distance separating the affected proton from the complexed molecule should be emphasized. Larger values of $\Delta\delta_c$ for a particular proton mean closer association with the lecithin. With equilibrium constants of similar magnitude, changes in chemical shift for the complex could be significant criteria for evaluating the degree of interaction of particular sites of the molecule. These values can vary considerably between different protons on the same molecule and between similar protons on different molecules.

It is clear, then, that these compounds can bind to lecithin. Present evidence suggests that the physiological response produced by DDT and related compounds depends on some type of interaction of these molecules in the membrane lipoprotein complex (1, 2). Different molecular models have been suggested, incorporating steric considerations (19–22), complex formation (3–7), electronic effects (23), etc. However, the basic questions as to the nature of this interaction are still unresolved. The interactions described in this study could be extended to natural systems only if the experimental environment or something comparable were known to exist in that system. Our present understanding of membrane structure precludes a definitive judgment in this regard. Thus the only alternative is to consider those structural features of the compounds which are important in lipid binding in relation to structural factors known to be important for biological activity—in this case, insecticidal activity.

If the benzylic proton of DDT is replaced by a chlorine or methyl group, insecticidal activity is lost. This could be a steric requirement or it could indicate a specific binding site. The large value of $\Delta\delta_c$ for the benzylic proton of p,p'-DDT indicates the degree to which this proton is involved in binding to lecithin. In contrast, DDE does not contain a benzylic proton, has little insecticidal activity, and does not bind to lecithin.

The extent to which a particular proton becomes involved in complex formation is clearly a function of its acidic character; for example, the $\Delta\delta_c$ values for the benzylic proton of p,p'-DDT and methoxychlor (Table 1). The benzylic proton of the latter is much less acidic in character. This is clearly demonstrated by the rates of base-catalyzed dehydrochlorination of these two compounds. p,p'-DDT reacts at a rate some 250 times as fast as methoxychlor (24). Sterically these two molecules are very similar, yet the DDT is generally more active as an insecticide. Thus one can draw a comparison between acidic character, lecithin binding, and biological activity.

In an intensive study of the stereochemistry of DDT and a series of related cyclopropane analogues Holan (21, 22) has defined the spatial requirements of their insecticidal activity. This analysis has been extended to include other series of compounds which are sterically equivalent, suggesting that these spatial requirements can be applied quite generally. Of signifi-

cance to this study is the fact that all those compounds shown to have insecticidal activity have a proton of acidic character located in the region of the molecule corresponding to the ethane moiety of DDT.

The proton on carbon 1 of DDD gave the largest $\Delta\delta_c$ values, reflecting the stronger inductive effect with the chlorine atoms on the same carbon as the proton. *p,p'*-DDD, although it produced a physiological response similar to *p,p'*-DDT in cockroach nerve (25), is much less active. Thus, if lipid binding is involved, the location of the proton in the interacting molecule could be a factor.

Studies with analogues of DDT have shown that insecticidal activity is lost (26) if substituents at the *para* position of the phenyl rings have either very positive (e.g., NO_2) or very negative (e.g., NH_2) Hammett *sigma* constants. It can be inferred from Holan's model (21, 22) that DDT-like compounds bind both to protein and lipid. If so, those substituents which enhance the π-electron density of the phenyl rings would facilitate binding to protein but have an adverse effect on lipid binding. The converse would be the case for those substituents which tend to withdraw electrons. Thus, if biological activity were expressed as a function of the electronic characteristics of the substituent groups in the aromatic ring (steric factors being constant), one would expect to observe maximum biological activity at some level of electronic interaction which gives a balance between these two opposing factors.

The interaction of *o,p*-DDT with lecithin was quite weak, in keeping with its generally weak insecticidal activity. In this case the availability of the benzylic proton must be restricted by the chlorine substituent at the *ortho* position. The binding characteristics of dicofol are quite distinct from the chloroethane compounds, as are its biological effects. It is assumed that the hydroxyl proton is involved in binding; however, this cannot be confirmed by NMR procedures.

Thus DDT-like compounds can bind to lecithin and comparisons can be made between binding characteristics and biological activity. To determine whether these relationships are significant will require further studies using a broader range of compounds and the extension of such investigations to systems which more closely approximate natural membranes.

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