MOLECULAR COMPLEXES OF 1,1,1-TRICHLORO-2,2-BIS (p-CHLOROPHENYL) ETHANE WITH AROMATIC DONORS

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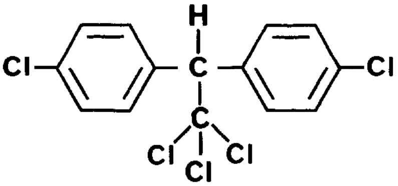
Received April 13, 1970

SUMMARY

A series of complexes between DDT acting as an electron acceptor and certain aromatic electron donors, including benzene, toluene, durene, mesitylene, pentamethylbenzene, and hexamethylbenzene, have been determined in solution using nuclear magnetic resonance spectroscopy. Variations in the proton chemical shifts between complexed and uncomplexed acceptor molecules are found to change relative to one another in the presence of excess donor. The sensitivity to small changes in the electrical environment of the magnetic nucleus of the acceptor molecule in the presence of donor compounds and the study of appropriate nuclear magnetic resonance parameters affords useful information concerning these complexes. Equilibrium constants for π-complex formation in $CHCl_3$ and CCl_4 have been determined by the Benesi-Hildebrand method or a modification of it

The effects on the public health of certain chlorinated hydrocarbons commonly used as pesticides, such as DDT, are of increasing interest. Many workers (1-4), in pointing out current ignorance about the mechanism of action of these pesticides, have stressed the need for studies aimed at establishing, on a molecular level, the basis of activity of the pesticides and their metabolites. Their toxicity is thought to be due to effects produced on the central nervous system of both vertebrates and invertebrates. DDT's mode of action *in vivo* has been studied by a number of investigators. Gunther *et al.* (5) proposed that



DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane)

Van der Waal's forces were involved in the binding of nerve protein with DDT, thus precipitating its toxic effects. Mullins (6) suggested that its toxicity may be due to its ability to fit into an inter-molecular lattice and act as a plug to prevent adequate ionic diffusion. Other workers (7) explain its toxicity in terms of disruption of proper balance of K^+ ion transport in the nervous system. A more specific postulate is that DDT selectively inhibits the action of an Na^+, K^+, Mg^{2+} - adenosine triphosphatase found in the nerve ending fraction of the rat brain (8). A recent, somewhat controversial, proposal for the mode of action of DDT is the charge transfer complex (π-complex) formation, a hypothesis of Matsumura and O'Brien (9). They have suggested that DDT may act by forming a charge transfer complex with protein components of the nerve axon, i.e., the formation of molecular complexes between electron donor (D) and electron acceptor (A) molecules by partial transfer of an electron from the donor to the acceptor. Although no causal relationship between the formation of charge transfer complexes and symptoms of poisoning has been established, the questions arising from this proposal prompted a further investigation of this assumption and collection of some quantitative data for π-complex formation of DDT with more common electron donors.

Certain molecular species are known to form π-complexes with aromatic and olefinic hydrocarbons (10, 11). The structure and chemistry of these complexes have been extensively investigated (12, 13), although only a few of the known complexes have been studied in detail. Visible, ultraviolet, and infrared spectroscopic techniques, and more recently, nuclear magnetic resonance (NMR) (14), have been useful in providing data about the structure of the complexes.

The method for obtaining equilibria data for π-complexes of this type, described by Benesi and Hildebrand (15), takes advantage of the fact that most π-complexes have a new absorption band in the visible or ultraviolet region of the spectrum which is characteristic of the complex itself. From plots of optical density versus donor concentration, the equilibrium constants and molar absorbancy index may be calculated.

A modification of the Benesi-Hildebrand equation (15) for determining equilibrium constants of this type has been derived for NMR applications. By considering the chemical shift of protons on molecules undergoing rapid exchange between complexed and uncomplexed states and by following treatments used in NMR studies of hydrogen bonding equilibria (16-18) the equation shown below may be derived.

$$\frac{1}{\Delta^{A}_{OBS}} = \frac{1}{K\,\Delta^{A}_{AD}\,c_{D}} + \frac{1}{\Delta^{A}_{AD}}$$

Δ^{A}_{OBS} is the observed shift of acceptor protons in the complexing medium; Δ^{A}_{AD} is the shift of acceptor protons in the pure complex; c_D is the concentration of donor in moles per kilogram of solvent; K is the equilibrium constant. This equation requires that $c_D >> c_A$. It also assumes that solutions are ideal or that the quotient $\frac{(\gamma AD)}{(\gamma A)(\gamma D)}$ remains constant over the range of solutions studied.

The utility of NMR in the study of π-complex formation has been demonstrated (14, 19, 20) by the close agreement between results obtained by the NMR method and those obtained by other spectroscopic techniques.

The observed chemical shifts of the benzylic proton of DDT upon addition of some aromatic donors in CCl_4 and $CHCl_3$ solutions are reported in this paper. These shifts are interpreted as resulting from charge transfer complex formation of DDT with these compounds. Some data concerning the structure of the complex itself and the magnitudes of the equilibrium constants of these complexes are also discussed.

EXPERIMENTAL

<u>Materials</u>: The DDT used was an analytical standard (98.9% purity) obtained from Aldrich Chemical Co. and used without further purification. All liquids were spectroquality reagents. Tetra-, penta-, and hexamethylbenzene were purified in the usual manner (21).

<u>Procedure</u>: A standard stock solution of DDT in both CCl_4 and $CHCl_3$ was prepared with the concentration just sufficient to observe an NMR signal for the benzylic

proton. A known amount of donor was weighed in a tared flask, 5 ml of DDT stock solution was added, and the flask was reweighed.

Instrumentation: The NMR spectra were recorded on a Varian Associates HA-100 high resolution spectrometer operating at 100 MHz. The line positions of the proton resonance were measured with a Hewlett Packard Model 5512 A electronic counter, which displays the pen position in cps relative to an internal tetramethylsilane (TMS) reference. Shifts were measured to an estimated accuracy of 0.25 Hz. All shifts in the signal caused by addition of electron donor to the system were to higher field values. Measurements were made at 36.2±1.0 °, the ambient temperature in the vicinity of the probe.

Equilibrium constants were calculated from the NMR data using a least squares regression program and a Wang Model 300 series electronic calculator.

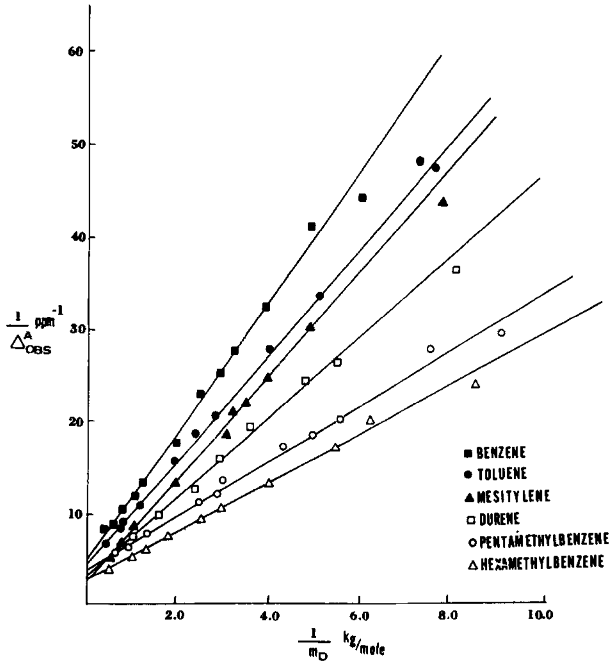


Fig. 1. Plots of $1/\Delta^A_{OBS}$ vs $1/m_D$ for the NMR shifts of the benzylic proton of DDT with aromatic donors in CCl_4 solution.

RESULTS AND DISCUSSION

Figures 1 and 2 show plots of the reciprocal of the observed shifts of acceptor protons versus the reciprocal of the concentration of donor molecules in CCl_4 and $CHCl_3$ solutions respectively. Good straight lines were obtained in all cases. The increased deviation at high values of $1/m_D$ is to be expected since this corresponds to low molalities of donor and to a breakdown of the approximation that $c_D >> c_A$. Tables 1 and 2 summarize the data for the range of donor concentrations, the observed shifts, the calculated values of equilibrium constants, and the chemical shifts of acceptor protons in the pure complex. The validity of the data in the tables is demonstrated by a plot of Δ_{OBS} vs m_D for the DDT-benzene system, Fig. 3. The conclusion can be drawn that K's determined from NMR measurements are valid as long as a plot of Δ_{OBS} vs the appropriate concentration gives a curved line (14).

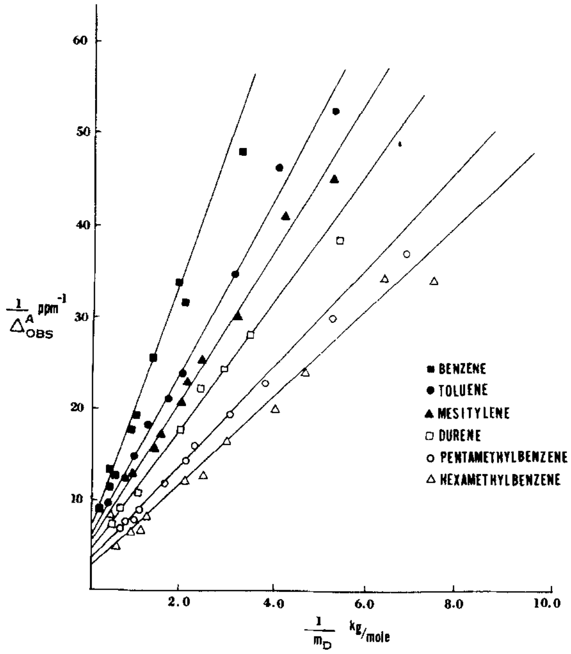


Fig. 2. Plots of $1/\Delta^A_{OBS}$ vs $1/m_D$ for the NMR shifts of the benzylic proton of DDT with aromatic donors in $CHCl_3$ solution.

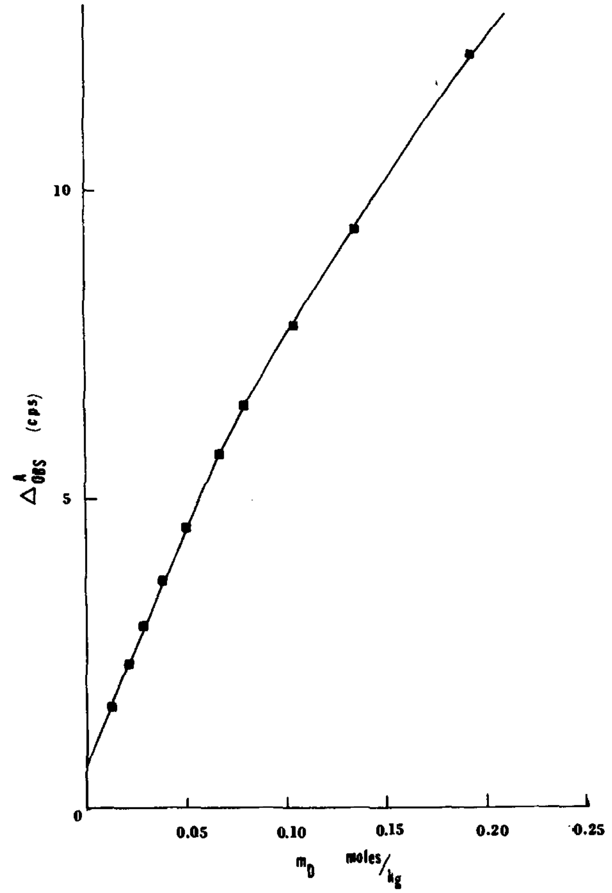


Fig. 3. A plot of Δ^A_{OBS} vs m_D for the NMR shifts of the benzylic proton of DDT with aromatic donors in CCl_4 solution.

Due to solubility the concentration range studied varied with each donor. As the concentration of donor was increased, an increase in the maximum observed shift was detected. Also, as the ionization potential of the donor increased, the observed shifts became smaller. Measurements to determine K were made by the acceptor methine proton resonance, and contributions to the observed values of the maximum observed shifts due to self-complexing are considered to be small. Since the complex can exist only in solution in equilibrium with donor and acceptor, the possibility of complex formation between the complex and another molecule (or another donor-acceptor species) was not determined.

The variations of K (Tables 1 and 2) in CCl_4 and $CHCl_3$ cannot be wholly

Table 1. A Summary of the Properties of π-Complex Formation of DDT with Aromatic Molecules in Carbon Tetrachloride Solution

Donor	Range of Donor Concentration (moles/kg Solvent)	Δ_{max} Observed (cps)	Δ^{A}_{AD} (cps)	K (kg/mole)
Benzene	0.05 - 3.5	17.0	24.5	0.034
Toluene	0.05 - 3.0	20.0	23.4	0.040
Mesitylene	0.05 - 2.0	17.0	35.8	0.065
Durene	0.10 - 0.80	28.0	60.2	0.135
Pentamethylbenzene	0.10 - 0.80	29.0	41.2	0.112
Hexamethylbenzene	0.10 - 0.50	20.0	52.6	0.189

Table 2. A Summary of the Properties of π-Complex Formation of DDT with Aromatic Molecules in Chloroform Solution

Donor	Range of Donor Concentration (moles/kg Solvent)	Δ_{max} Observed (cps)	Δ^{A}_{AD} (cps)	K (kg/mole)
Benzene	0.05 - 3.5	18	18.2	0.013
Toluene	0.05 - 3.0	18	18.5	0.019
Mesitylene	0.05 - 2.0	25	22.4	0.027
Durene	0.10 - 0.80	17	27.5	0.034
Pentamethylbenzene	0.10 - 0.80	28	26.9	0.050
Hexamethylbenzene	0.10 - 0.50	18	33.0	0.060

attributed to the dielectric constant, refractive index, or other physical properties of the solvent. Probably the most important cause for the discrepancies in the values of K in the two solvents is the competition of solvent for donor molecules. This effect is greater for $CHCl_3$ than for CCl_4, and may account for the smaller values obtained for K in $CHCl_3$ (22).

We observed no definite trends in the values of the shift of the acceptor protons in the pure complex with the structure of the donor in comparing our results with values obtained by previous workers (14, 20). The same magnitude of differences in the shift of the protons of pure complex is obtained for the same complex in CCl_4 and $CHCl_3$. It must be noted that absolute magnitude of the observed shift of acceptor protons is a function only of the structure of the actual complex and should be independent of the concentration scale.

Several observations may be used to support the assumption that these observed shifts for complex formation of DDT with the aromatic donors are due to molecular complexing. The equilibrium constants are in the right order, i.e., as the ionization potential becomes lower, the equilibrium constant increases. When DDT was replaced with toluene no shift of the toluene protons was observed. These observations strongly suggest that the observed shifts of the benzylic proton of DDT are due not to some kind of general solvent effect but to molecular complexing.

In determining equilibrium data, the major advantage of NMR over other spectroscopic techniques is that is is possible to study complexes that have no separate charge transfer band in the visible or ultraviolet spectrum, or where the charge transfer band is obscured by the spectrum of one of the other components. Also, it is easier to measure line position than an intensity (23). The system investigated in this work was ideal for NMR study in several ways: (1) The benzylic proton of DDT affords a single sharp resonance line at approximately 495 Hz. (2) This absorption peak does not overlap with the absorption peaks of the donor molecules studied, making it easy to measure its position. (3) We were able to make the donor concentration large with respect to the acceptor molecule, DDT. (4) The absorption peak observed did not overlap with the absorption peaks of the two solvents used in this study. Since it is difficult to find an experimental system in which the same or similar conditions are true, care must be taken in choosing the model system for study of complex formation by this technique.

This work has demonstrated the existence of charge transfer complexes of DDT in nonaqueous solution. We are engaged in a variable temperature study of this system to obtain some data for the bond energies and other physical constants for these complexes. Also, we are searching for a suitable solvent with which to extend the use of this method to further investigate the complex formation of DDT with suitable biological substrates *in vitro* as a possible approach to determining the mode of action of this compound.

REFERENCES

(1) Calhoun, E.H., J. Agr. Food Chem., 8, 252 (1960).
(2) O'Brien, R.D., "Insecticides: Action and Metabolism," Academic Press, N.Y., 1967, p 108.
(3) Hennessy, D.J., Boyce-Thompson Inst., 8, 481 (1962).
(4) Abedi, Z.H., Botyu-Kagaku, 25, 156 (1960).
(5) Gunther, F.A., Blinn, R., Carmen, G.E., and Metcalf, R.L., Biochem.-Biophys., 50, 504 (1954).
(6) Mullins, L.J., Science, 122, 118 (1955).
(7) Yamasaki, T., and Narahashi, T., Botyu-Kagaku, 22, 296 (1957).
(8) Matsumura, F., and Patil, K.C., Science, 166, 212 (1969).
(9) Matsumura, F., and O'Brien, R.D., J. Agr. Food Chem. 14, 38 (1966).
(10) Mulliken, R.S., J. Am. Chem. Soc., 72, 600 (1950); 74, 811 (1952); J. Phys. Chem., 56, 801 (1952).
(11) Andrews, L.J., Chem. Rev.,54, 713 (1954).
(12) McGlynn, S.P., Chem. Rev.,58, 1113 (1958).
(13) Keefer, R.M., and Andrews, L.J., J. Am. Chem. Soc., 74, 640 (1952).
(14) Hanna, M.W., and Ashbaugh, A.L., J. Phys. Chem., 68, 811 (1964).
(15) Benesi, H.A., and Hildebrand, J.H., J. Am. Chem. Soc., 71, 2703 (1949).
(16) Berkeley, P.J., Jr., and Hanna, M.W., J. Phys. Chem., 67, 846 (1963).
(17) Huggins, C.M., Pimentel, G.C., and Shoolery, J.N., J. Chem. Phys., 23, 1244 (1955).
(18) Becker, E.D., Liddel, U., and Shoolery, J.N., J. Mol. Spectry., 2, 1 (1958).
(19) Schug, J.C., and Martin, R.J., J. Phys. Chem., 66, 1554 (1962).
(20) Foster, R., and Fyfe, C.A., Trans. Faraday Soc., 61, 1626 (1965).
(21) Foster, R., and Hanson, P., Trans. Faraday Soc., 60, 2189 (1964).
(22) Thompson, C.C., Jr., and DeMaine, P.A.D., J. Am. Chem. Soc., 85, 3096 (1963).
(23) Hood, G.C., Redlich, O., and Reilly, C.A., J. Chem. Phys., 22, 2067 (1954).