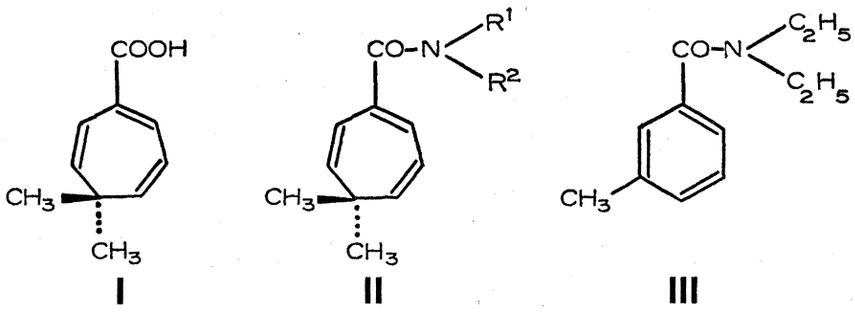

Fig. 1. Structures of thujic acid **I**, the amides of thujic acid **II**, and *N,N*-diethyl-*m*-toluamide **III**. Substituent $R^1$ and $R^2$ groups for **II** are identified in Table 1.

are crystalline solids. Elemental analysis, gas-liquid chromatography, and infrared spectroscopy confirmed the identity and purity of the compounds prepared and submitted for testing. Only the amide and the anilide of thujic acid have been prepared previously. Neither of these compounds showed any type of biological activity (*5*).

The candidate compounds were screened and evaluated at the Entomology Research Division, U.S. Department of Agriculture, Beltsville, Maryland (*6*), and at the Insect Pathology Research Institute, Department of Fisheries and Forestry, Sault Sainte Marie, Ontario (*7*). Brief reference is made here to part of the results obtained at the former institution.

In repellency tests carried out with *Aedes aegypti*, individuals exposed their arms covered with cotton stockings, treated with 3.3 g of the screened compound per square foot (0.093 $m^2$) of stocking, for 1 minute at intervals of from 1 to 7 days in cages containing mosquitoes. The measure of the repellency was given by the number of days with lasting protection until five mosquito bites occurred in a 1-minute period. Under these conditions the diethylamide **IId** was effective for 50 days, whereas the standard repellent dimethyl phthalate is effective for 11 to 22 days. The exceptionally active compound 2-butyl-2-ethyl-1,3-propanediol is effective for 196 days (*8*).

Repellency screening with American and German cockroaches was carried out with two cardboard boxes, one treated with 1 ml of a 1 percent acetone solution of the tested compound and the other a blank. Each of the boxes had a volume of 82 ml. Ten male and ten female insects were given the choice of entering either box. The duration of the experiment was 1 week. Six times each day at regular intervals the number of insects in each box was counted. After each count the insects were shaken out, and the positions of the boxes were reversed. The measure of repellency was given by the percentage of insects in the treated and untreated box, respectively. Fencholic acid, a standard repellent, was tested concurrently with the candidate repellent. In these tests the *N,N*-diethylamide **IId** was at least 35 percent more active than fencholic acid.

The evidence presented here seems to suggest that a prerequisite for repellent activity in this class of compounds appears to be dialkyl substitution on the amide nitrogen. The *N,N*-dimethylamide **IIb** exhibited repellent activity against mosquitoes for 1 day but failed to repel cockroaches. The *N,N*-dibutylamide **IIf** had about 25 percent of the effectiveness of fencholic acid in repelling *Blatella germanica* but was inactive against *Aedes aegypti*. The aromatic, heterocyclic, and carbocyclic amides **IIg** through **II**$\ell$ were inactive, as were the *N*-monoalkylamides **IIa**, **IIc**, and **IIe**. Both the *N*-monoethylamide **IIc** and the *N*-monocyclohexylamide **IIg** showed a distinct degree of attractancy toward *Aedes aegypti*. Unfortunately, the extreme difference in volatility between **IIc** as well as **IIg** and the standard attractant L(+)lactic acid does not allow for a more quantitative comparison.

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1. For a pertinent summary and a list of leading references see: R. L. Metcalfe, in *Encyclopedia of Chemical Technology*, A. Standen, Ed. (Interscience, New York, ed. 2, 1966), vol. 11, pp. 723–738.
2. H. McLean, *Forest. Prod. J.* **20**, 48 (1970), and references therein.
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4. The chloride of thujic acid was prepared by the *N,N*-dimethylformamide-catalyzed reaction of the acid with thionyl chloride at room temperature. The 50 mole percent excess of $SOCl_2$ used in the reaction was subsequently distilled off, and the crude chloride was used directly. Thujic acid is known to isomerize under acidic conditions of *p*-isopropylbenzoic acid. In a set of control experiments we ruled out the occurrence of this rearrangement under our experimental conditions.
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6. We thank Dr. M. Beroza, Dr. C. H. Schmidt, S. A. Hall, D. E. Weidhaas, and their collaborators for screening our samples and for permission to use the resultant data in this report.
7. We appreciate the cooperation of Dr. J. Weatherston.
8. Our work was originally initiated on the basis of an analogy between amide **III** and the projected amides **II**. However, the results presented here relate to standard compounds that are more specific repellents for insect species used for the screening tests described.
9. Work was partly supported by the National Research Council of Canada. We acknowledge permission to publish this report given by MacMillan Bloedel Research, Ltd.
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## Binding of DDT to Lecithin

Abstract. *An interaction between DDT and lecithin is indicated by the reciprocal effects of each compound on the proton magnetic resonance spectrum of the other. The phosphoryl choline moiety of the lecithin and the benzylic proton of the DDT seem to be involved. The most pronounced response in the proton magnetic resonance spectrum of the lecithin produced by increasing concentrations of DDT was a change in the chemical shift of the resonance peak due to the protons of the choline methyl groups. Increasing concentrations of lecithin produced changes in the chemical shift of the resonance peaks of the benzylic proton and adjacent ring protons of the DDT. Equilibrium constant of 0.597 ± 0.015 molal$^{-1}$ was obtained for this interaction.*

The toxicity of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] is generally attributed to its effect on the central nervous system where it produces an excitatory effect on axons. In an effort to define the molecular basis of the response, Matsumura and O'Brien have demonstrated that DDT will bind to components of cockroach nerve (*1*). Using spectral and fluorescence data these workers have postulated the formation of a charge-transfer complex. This hypothesis is open to criticism because comparable spectral changes can

Table 1. Changes in the chemical shift of benzylic and aromatic protons of DDT (0.009 molal) by the addition of lecithin in $CCl_4$ at 33°C.

| Lecithin concentration (molal) | Chemical shift (hz) | | |
|---|---|---|---|
| | Benzylic proton | Ring proton b | Ring proton c |
| 0 | 495.2 | 746.0 | 726.3 |
| 0.072 | 497.6 | 747.3 | 726.4 |
| .123 | 499.2 | 747.6 | 726.5 |
| .156 | 500.1 | 747.9 | 726.5 |
| .17 | 501.8 | 748.3 | 726.9 |
| .25 | 502.6 | 748.9 | 726.7 |
| .293 | 503.9 | 749.3 | 726.7 |
| .332 | 504.7 | 749.6 | 726.9 |
| .368 | 506.2 | 749.9 | 726.7 |

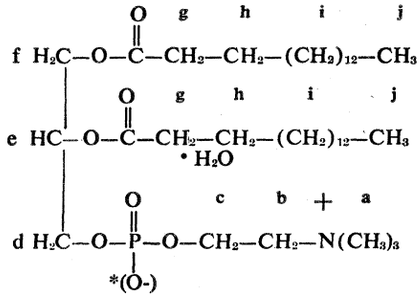
be obtained if solutions of DDT stand at room temperature (*2*). In this instance the spectral changes have been attributed to intermolecular charge-transfer complexing of the DDT. Wilson and coworkers (*2*) have shown, however, that DDT can act as a relatively weak electron donor in forming a charge-transfer complex with tetracyanoethylene. Changes in the chemical shift of the benzylic proton produced by aromatic donors have also been attributed to charge-transfer complexing. In this instance the DDT would be presumed to be acting as an acceptor (*3*).

In another way, Hilton and O'Brien (*4*) have shown that DDT will block the action of valinomycin on a phospholipid bilayer. Addition of this cyclic antibiotic to a lecithin-decane bilayer produces an increase in conductance across the bilayer, and this response is blocked by the subsequent addition of DDT. In this respect DDT differed from lindane and dieldrin. Whether the DDT interacted with the valinomycin, the lipid, or both constituents is not yet apparent.

To define the molecular basis for the action of DDT will require further information on the nature of its interaction with the component molecules of affected systems. One obvious omission at present is the lack of data concerning the interaction of DDT with complex lipids. We now discuss the interaction of lecithin and DDT as it is defined by nuclear magnetic resonance spectroscopy.

Proton magnetic resonance (PMR) spectra of lecithin and DDT in $CHCl_3$ or $CCl_4$ were recorded on a Varian HA 100-Mhz spectrometer, with tetramethylsilane (TMS) as an internal lock standard. The chemical shifts were determined with a precision of ± 0.25 hz. The β,γ-dipalmitoyl-DL-phosphatidylcholine monohydrate (Sigma) was used in these experiments as obtained, without further purification. Reagent grade DDT (City Chemical) and spectrograde chloroform and $CCl_4$ were used.

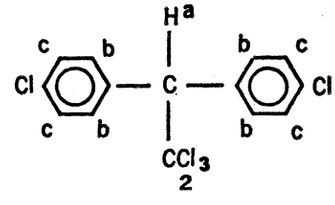
The PMR spectrum of this particular lecithin has been described (*5*). As was expected from the structure of this compound (**1**), the spectrum gave peaks with varying degrees of multiplicity which corresponded to protons a to i. In addition, the PMR spectrum always showed a peak whose chemical shift was dependent on the concentration, the




**1**

temperature, and the solvent. This peak was attributed to a condensed water molecule associated with the lecithin. Addition of DDT (0.770 molal) to a lecithin solution (0.045 molal) in $CHCl_3$ produced low field changes in the chemical shift of various lecithin protons. The $-N(CH_3)_3$ protons peak showed the maximum change in chemical shift (~11 hz), whereas proton b was shifted 5 hz. Other lecithin protons also showed very small low field changes in chemical shift (~2 hz). However, the position of the terminal methyl proton j was unaffected. The associated water proton peak also gave a low field shift.

The PMR spectrum of DDT (**2**) in $CHCl_3$ or $CCl_4$ showed a sharp peak due to the benzylic proton "a" at approximately 5.0 parts per million (*6*). The ring proton




**2**

spectrum showed a complex pattern because of ortho and meta spin splitting between the magnetically nonequivalent ring protons (*6*). Addition of the lecithin to a solution of DDT produced low field changes in the chemical shift of the benzylic proton.

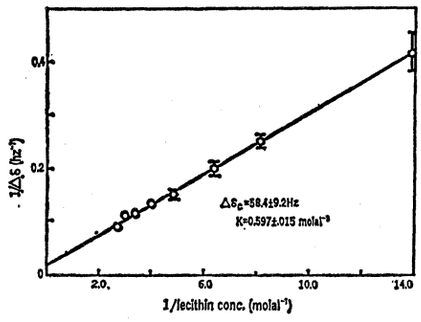



Fig. 1. Reciprocal plot of change of chemical shift of benzylic proton of DDT as function of lecithin concentration at 33°C.

The ring protons "b" showed similar though much smaller changes in chemical shift. No appreciable change in the chemical shift of the ring protons "c" was observed (Table 1).

These induced changes in chemical shift would suggest an interaction between lecithin and DDT. Apparently the rate of proton exchange between the lecithin-DDT complex and the reactants is very rapid on the nuclear magnetic resonance time scale, and only one average peak is observable.

The equilibrium for the binding of DDT to lecithin can be expressed as

$$(\text{Lecithin}) + (\text{DDT}) \rightleftharpoons (\text{Lecithin}) \cdot (\text{DDT}) \quad (1)$$

The equilibrium constant $K$ can be calculated with the use of the following expression (*7*)

$$\frac{1}{\Delta\delta_0} = \frac{1}{K\Delta\delta_c C} + \frac{1}{\Delta\delta_c} \quad (2)$$

where $C$ is the concentration of lecithin and $\delta$ is the chemical shift in parts per million. $\Delta\delta_0$ is the difference between $\delta$(observed) and $\delta$(noncomplexed DDT) and $\Delta\delta_c$ is the difference between $\delta$(complexed DDT) and $\delta$(noncomplexed DDT). The above relation (Eq. 2) is valid only when the concentration of DDT is much less than that of the lecithin. If $1/\Delta\delta_0$ is plotted as a function of $1/C$ (Fig. 1) and a least squares fit is used, $\Delta\delta_c$ is calculated to be $58.4 \pm 9.2$ hz and $K$ to be $0.597 \pm 0.015$ molal$^{-1}$.

The inductive effect of the three chlorine atoms would increase the acidic character of the benzylic proton which, consequently, would be more likely to associate with an electronegative atom such as the oxygen bound to the phosphorus. The change in electronic environment produced by such an interaction could account for the observed changes in the chemical shift of the benzylic proton and the ring protons,

b. The ring protons, c, are further removed from the site of interaction and would not be expected to respond to the same degree.

Transmitted changes in the electronic environment resulting from this type of interaction would account for the changes in chemical shift of the lecithin protons. The more pronounced changes in the chemical shift of the *N*-methyl protons could result from their proximity to the interacting molecule. Normally one would expect the phosphorylcholine moiety to assume a conformation allowing interaction of the quaternary nitrogen and the negative charge of the phosphate.

The effect of the DDT on the resonance peak of the protons of the associated water molecule depends on the initial concentration of the lecithin. At concentrations higher than 0.018 molal of lecithin, a low-field change in the chemical shift of lecithin is observed by the addition of DDT. In more dilute solutions of lecithin, however, DDT produced significant line broadening with only small changes in chemical shift.

The potential for such an interaction in biological systems will depend on the nature of the environment of the lecithin molecule in a membrane, and this situation has not yet been defined. However, a rather strong association can occur between lecithin and DDT, and further studies in more complex systems should provide some indication of the significance of this interaction. The involvement of the benzylic proton of the DDT is of significance, and this observation would substantiate observations of Ross and Biros (*3*).

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4 May 1971; revised 28 June 1971

# Xeroderma Pigmentosum: A Rapid Sensitive Method for Prenatal Diagnosis

Abstract. *When normal human cells, capable of repairing ultraviolet-induced lesions in their DNA, are incubated in the thymidine analog 5-bromodeoxyuridine after ultraviolet irradiation, the analog is incorporated into the repaired regions. When such repaired cells are subsequently irradiated with 313-nanometer radiation and placed in alkali, breaks appear in the DNA at sites of incorporation of 5-bromodeoxyuridine, inducing a dramatic downward shift in the sedimentation constant of the DNA. Cells from patients with the disease xeroderma pigmentosum, which causes sensitivity to ultraviolet, are incapable or only minimally capable of repair; such cells incorporate little 5-bromodeoxyuridine into their DNA under these conditions and, upon 313-nanometer irradiation and sedimentation in alkali, exhibit only minor shifts in DNA sedimentation constants. When fibroblasts developed from biopsies of normal skin and of skin from patients with xeroderma pigmentosum, as well as cells cultured from midtrimester amniotic fluid, were assayed in this fashion unequivocal differences between normal and xeroderma pigmentosum cells were shown. Xeroderma pigmentosum heterozygotes are clearly distinguishable from homozygous mutants, and results are available 12 hours after irradiation.*

A method for prenatal diagnosis for a genetic defect should be unequivocal and reasonably rapid so that a therapeutic abortion, if indicated, can be performed as soon as possible. In the case of the rare autosomal recessive human disease xeroderma pigmentosum (XP), no prenatal diagnostic test having these characteristics has previously been described. Any decision to perform an abortion is best made after consideration of the results of the method outlined below, other analyses of the repair capabilities of amniocentetic cells, and medical and genetic analysis of the family.

Xeroderma pigmentosum is characterized by extreme sensitivity to sunlight resulting in changes in skin cells which eventually lead to multiple actinic carcinomas (*1*). When XP cells are examined for DNA repair after ultraviolet irradiation they show a decrease or absence of unscheduled synthesis (*2*), decrease or absence of repair replication (*2*), and no excision of pyrimidine dimers induced by ultraviolet (*3*). These phenomena reflect the apparent molecular basis of the disease —lack of a functional form of the ultraviolet endonuclease (*3*, *4*). However, the three phenomena are less satisfactory as tests for normal or abnormal repair in fetal cells derived from transab-

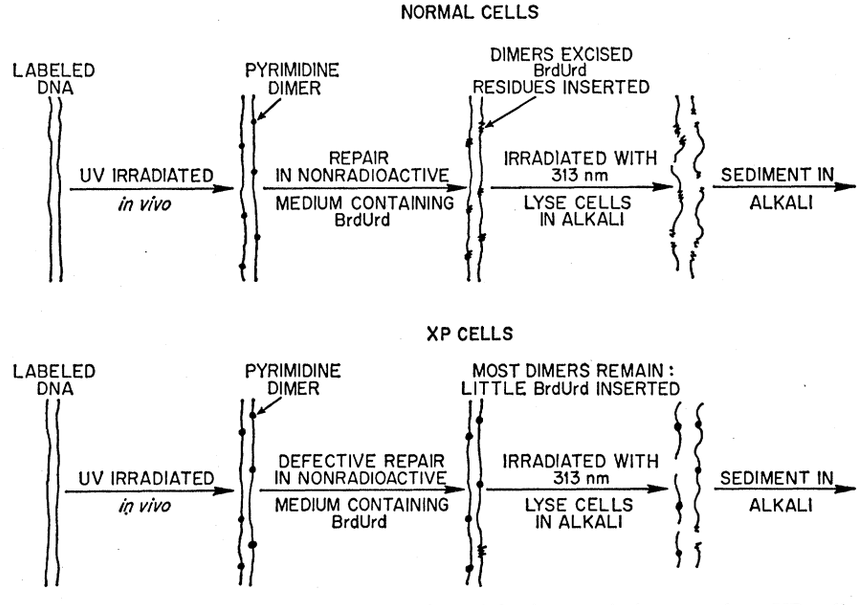


Fig. 1. Scheme for the detection of normal or defective repair in normal or XP cells. For control purposes, both cell types are incubated in nonradioactive medium containing thymidine rather than BrdUrd.