complex carbohydrates of certain biological fluids might be released from the corresponding cell surfaces[1,37,38].

*Resumen.* Se estudió el contenido de hexosas, hexosaminas, metilpentosas y ácido siálico de una fracción de membrana plasmática del epitelio de transición de la vejiga de cordero. La concentración de ácido siálico y hexosaminas era 3 y 5 veces respectivamente más alta en membrana plasmática que en homogenato total. En cambio, los carbohidratos neutros no se enriquecieron significativamente. El 23% de ácido siálico se extrajo con los lípidos de esa fracción. Se presentan datos de cromatografía y electroforesis.

N. IBAÑEZ, A. CANDIOTTI, R. O. CALDERON and B. MONIS

*Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla Postal 362, Córdoba (Argentina), 28 November 1973.*

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## Mode of Action of DDT Analogues: Molecular Orbital Studies

In earlier work a steric theory of DDT action has been used to design a large number of highly active DDT analogues[1,2]. The basis of this theory is that a molecule of an active compound has to form a 'molecular wedge' with two distinct features. Firstly, the apex of the 'wedge' (e.g. $CCl_3$ group of DDT) must have a particular size and shape, and secondly, the base comprises a multiple ring structure which must have electron donating substituents. The size limitation on the base part of the molecule is less restrictive than that on the apex.

It has been found that compounds synthesized to fit this model, while all possessing insecticidal activity, nevertheless exhibit considerable variations of this activity even in strictly standardized insect strains.

Recently, FUKUTO et al.[3] examined the structure/activity correlation for a series of DDT trichloroethane analogues by means of a multiple regression analysis using empirical substituent constants and mortality values in the housefly and *Culex* mosquito larvae. They obtained good correlations between log $LD_{50}$ and a function of the steric substituent constant $E_s$.

In this preliminary note we report the first results of an alternative method of correlating the activity of DDT analogues of a similar chemical structure with other molecular properties. We assume that any differences in this activity can be attributed to differences in electronic structure as the molecules have all been selected according to rigorous steric criteria.

All our insect mortality data were obtained[1] on the susceptible strain (WHO/IN/1) of houseflies which were selected by sex, age and weight of pupae. The flies were kept at standard humidity and temperature and fed on cellulose free of traces of insecticides. Other controlled variables were the time of day of application of the insecticides and the time of mortality counting. The mortality calculations were carried out only for tests where control mortality was zero for the 48 h test period. The compounds were potentiated with a mixed function oxidase inhibitor (Sesoxane) to obtain the basic activity comparison without superimposed biochemical degradation of the insecticides.

To estimate the charge distribution on the molecules we have performed molecular orbital calculations at the CNDO/2 level. This method is suitable for the calculation of charge distributions of molecules[4]. The computer program we used was that published by POPLE and BEVERIDGE[5] extended to take molecules with 55 atoms and 125 valence electrons and modified slightly to run under the highest optimization available on the CD 6600 computer.

The equation relating activity to charge distribution is based on the method of CAMMARATA[6], and a similar equation has been successfully used by NEW and RICHARDS[7] in a study related to ours, the molecular orbital calculation of hapten-antibody interactions. Since we are assuming that all steric factors are constant, we have[6]

$$\log LD_{50} = k \sum_i \frac{q_{s_i}\, q_r}{D_{rs_i}} + C.$$

[1] G. HOLAN, Nature, Lond. *221*, 1025 (1969).
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Relationship between calculated charges on chemically unrelated DDT-type insecticides and mortality in a standardized strain of the housefly

| Compound | $LD_{50}$ (μg/♀ insect) | Charge on apex of molecules ($10^{-4}$ × charge on an electron) |
|---|---|---|
| 1,1-*Bis*-(*p*-ethoxyphenyl)-2,2-dimethyl propane | 0.32 | + 210 |
| 1,1-*Bis*-(*p*-chlorophenyl)-2,2-dichloro cyclopropane | 0.12 | − 325 |
| 1,1-*Bis*-(*p*-ethoxyphenyl)-2-nitropropane | 0.065 | − 757 |
| 1,1-*Bis*-(*p*-ethoxyphenyl)-2-nitrobutane | 0.061 | − 679 |
| 2,2-*Bis*-(*p*-ethoxyphenyl)-3,3-dimethyl oxetane | 0.01 | −1776 |

where the $q_{s_i}$ are charges on the insecticide atoms, $q_r$ is the charge on the receptor, $D_{rs_i}$ the distance between charges, $k$ a constant which includes the reciprocal of the dielectric constant, and Ca constant taking into account interactions other than the electrostatic binding. We have evidence[2] that the compounds all act at the sodium channel of a nerve membrane but no detailed information concerning the receptor charge distribution. We therefore approximate the above equation by assuming that all receptor factors are constant. Thus

$$\log \mathrm{LD}_{50} = \mathrm{A} \sum_i q_{s_i} + \mathrm{C} \ ,$$

where $A$ is assumed constant. Now $\sum_i q_{s_i} = 0$ for neutral molecules if the summation is taken over all the atoms. However, since the model postulates two distinct parts of the 'molecular wedge' we have taken the sum over the part containing the two aryl rings and the bridging atom only and placed the balance of the charge on the apex. The results are summarized in the Table. A straight line of best fit through the points has a correlation coefficient of 0.998.

This finding is in accord with the effects[2] of substitution in the aryl rings on the negative coefficient of temperature with insect mortality. This was found to be independent of the chemical nature of the apex of the insecticide, for compounds of similar steric features which fit our model.

We consider the results of this work promising enough to continue calculations with more compounds and more accurate molecular orbital methods.

*Zusammenfassung.* Neues Verfahren mittels Molekularorbital-Berechnung zur Vorausermittlung der chemischen Konfiguration und Wirksamkeit von Insektiziden vom Typ des DDT.

G. Holan and T.H. Spurling[8]

*CSIRO Division of Applied Chemistry, P.O. Box 4331, Melbourne (Victoria 3001, Australia), 5 November 1973.*

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## A Study of the Combined Raman and Fluorescence Scattering from Human Blood Plasma

Raman spectroscopy has experienced a revival during recent years due to the utilization of lasers for excitation. It has thus been possible to extend this technique to studies on the conformation of complex organic molecules in aqueous solutions, such as proteins (Lord and Yu[1]). It seemed therefore possible that new information on the physical structure of the dominating constituents of blood plasma might be obtained. In connection with such studies we observed that the overlapping fluorescence scattering shows features which seem to be of clinical importance.

The weak intrinsic fluorescence of blood plasma, which is of the same order of magnitude as the Raman scattering, has not been examined earlier, so far as we know. Laser light excitation at different wavelengths were used, and we found that the combined fluorescence and Raman spectra in the region 542 nm –648 nm are particularly interesting. The spectra are thus changed in a regular way at various diseases, as will be described below.

Plasma samples from patients suffering from different organic diseases with definitely settled diagnoses were analyzed: lues, erysipelas, mycosis fungoides, viral hepatitis, sepsis, leucemia, and advanced carcinoma with methastases and different primary site. Healthy controls of both sexes covering all age groups were also analyzed. After an overnight fast of at least 12 h, samples of venous blood were taken. The blood was collected in standard heparin tubes (Vitrum). After centrifugation for 10 min at 2500 *g*, 0.1 ml plasma was injected into a glass capillary, diameter 0.1 mm, by careful avoiding formation of air bubbles.

A Cary 82 spectrophotometer with an argon laser was used. The capillary was adjusted in the centre of the unfocused green line (514.5 nm), with the axis of the capillary parallel with the beam. It was tedious work to adopt the experimental running conditions, so that reproducible spectra could be obtained. The laser power finally used, measured at the sample, was 150 mW. Fluorescence maxima and broad Raman bands, such as

[1] R. C. Lord and N.-T. Yu, J. molec. Biol. *50*, 509 (1970).

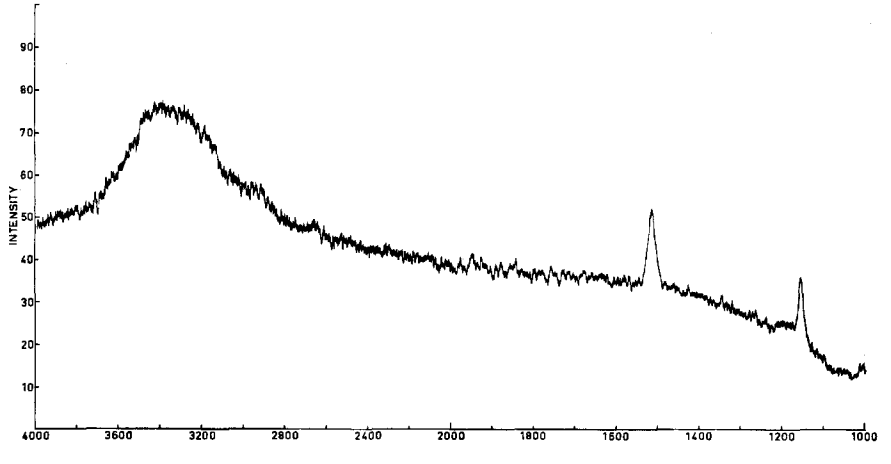


Fig. 1. Spectrum from a healthy woman, age 23. No history of any disease. Routine hematological and urinary analyzes normal.