Partition of an Organophosphorus Compound, Dichlorvos, between Liquid and Liquid Crystalline Phases

STIG FRIBERG *x **and LENNART LARSSON** ‡

Abstract □ The partition coefficients for an organophosphorus compound, dichlorvos (2,2-dichlorovinyl dimethyl phosphate), between pure liquids, micellar solutions, and liquid crystalline phases were determined by spectrometric analysis of the dichlorvos content after equilibration and separation of the phases. The results demonstrated the pronounced importance of associated colloid structures on the partition coefficient and showed that the partition coefficient did not display a simple concentration dependence.

Keyphrases □ *Dichlorvos—partition coefficients between pure liquids, micellar solutions, and liquid crystalline phases, effect of associated colloid structures* □ Partition coefficient of dichlorvos—determination in pure liquids, micellar solutions, and liquid crystalline phases, effect of associated colloid structures □ Organophosphorus compounds—partitioning of dichlorvos between liquid and liquid crystalline phases, effect of associated colloid structures

Many organophosphorus compounds are potent inhibitors of acetylcholinesterase and some are widely used as insecticides (1, 2). The most active members of the group are considered to be potential warfare agents (3). In addition to enzyme affinity, two other factors are important for the inhibitory efficiency of organophosphorus compounds: *(a)* the partition coefficients between different tissues with varying hydrophilic–lipophilic character (4), and *(b)* the metabolic degradation, mainly by hydrolysis, which causes detoxification (5).

Organophosphorus inhibitors have been found to show an unexpectedly high penetration into axons (6) in spite of having oil–water partition coefficients of only 0.1 (7). This fact has been considered a reason for the unexpected pharmacological response (8).

The purpose of this article is to draw attention to another factor of importance for the penetration of a drug in addition to its hydrophilic–lipophilic balance. Biological tissue is not, in general, a simple solution; the cellular membranes and the myelin part of the nerve substance are highly ordered systems with regular layer structures. Such structures (9) can be compared with the structure of a liquid crystalline phase (10). Liquid crystalline phases may actually assume many structures (11) (Fig. 1); the transition from a layer structure to a regular hexagonal packing of cylinders has been suggested as being responsible for the transport mechanism through membranes (12).

Since liquid crystalline structures appear to be of general importance for biological systems and since organophosphorus compounds have been found to penetrate axons in spite of low oil–water partition coefficients (7), an investigation of the possible effects on the partition of structuring elements such as

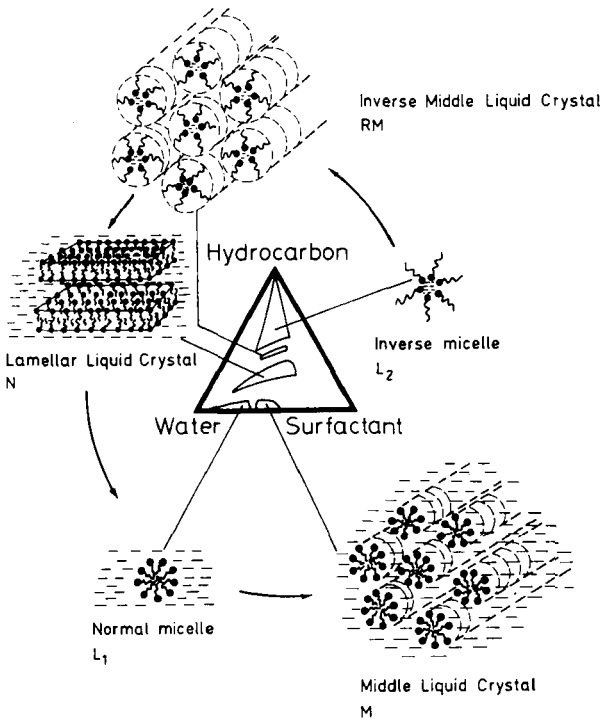


Figure 1—*When water, a hydrocarbon, and an amphiphilic compound are combined, micellar solutions with normal (L_1) and inversed (L_2) micelles and liquid crystalline phases [middle liquid crystal (M), lamellar liquid crystal (N), and inversed middle liquid crystal (RM)] are obtained.*

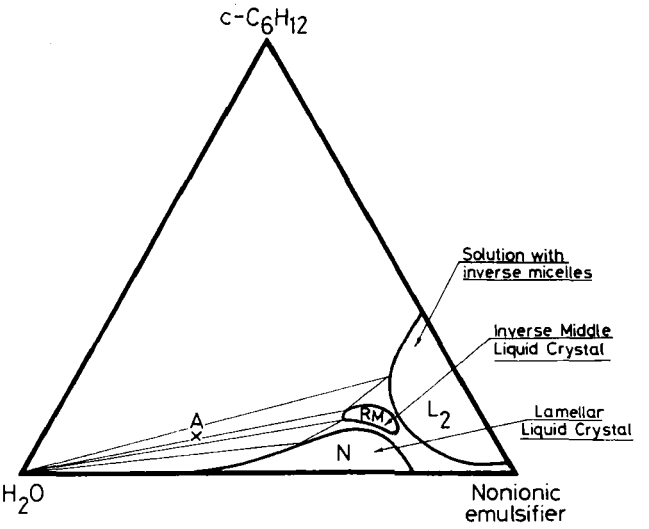


Figure 2—*Water, cyclohexane, and the nonionic emulsifier pentaoxyethylene dodecyl ether formed three liquid phases [pure water, pure cyclohexane, and a solution of cyclohexane in the emulsifier (L_2)] and two liquid crystalline phases [lamellar phase (N) and inversed middle phase (RM)].*

micelles in liquids and of the general longrange order characteristics of a liquid crystalline phase was considered to be of value. No earlier investigation of the distribution of organophosphorus compounds between phases containing associated structures was found in the literature. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) was selected for the investigation because its properties are well known (13) and its solubility in hydrocarbons and water is balanced.

Two different systems containing associated colloids were selected. In addition to water and cyclohexane, the first one contained two different liquid crystalline phases (RM and N) and one solution (L_2) with inverse micelles (Fig. 2). These three phases displayed comparatively small differences in their total composition and were judged well suited for the purpose of estimating the influence of different associated structures. The second system contained an alkylammonium carboxylate (Fig. 3) and was used to observe the influence on the partition of a gradual increase in the amount of associated structure units.

EXPERIMENTAL

Materials—The dichlorvos used was a commercial sample[1], which was purified by distillation with a spinning-band column (13 torr, 112°). Double-distilled water, cyclohexane, *p*-xylene, 1-aminooctane[2], 1-octanoic acid[3], and a commercial pentaoxyethylene dodecyl ether were used without further purification.

Separation of Phases—Between 0.5 and 1.5 wt. % of dichlorvos was dissolved in the mixture of water, hydrocarbon, and surfactant. The mixture was heated to 30° and allowed to cool slowly to 20°, at which temperature it was allowed to equilibrate for 24 hr. Centrifugation with an analytical centrifuge[4] (36,000 rpm for 16 hr) caused the phases to separate from each other.

Analysis—The system consisting of water, cyclohexane, and nonionic emulsifier did not show UV absorption at a level that would influence the determination of the dichlorvos at 205–207 nm. Separate calibration curves were determined for each phase involved in the equilibrium by addition of known amounts of dichlorvos to the separated phases.

For the system consisting of water, *p*-xylene, octanoic acid, and aminooctane, the determination of the amount of dichlorvos was done spectrophotometrically on the antimony phosphatomolybdate complex (14) after the phosphorus had been transformed to phosphate using sulfuric and nitric acids. Separate calibration curves also were determined for the different phases.

RESULTS

The regions for the different phases in the water–cyclohexane–surfactant system are given in Fig. 2. The nonionic surfactant used for the first system was soluble to less than 0.5% in water or in cyclohexane. In Fig. 2, the liquid phases of water and cyclohexane are only marked as points in the respective corners. Both water and cyclohexane were soluble in the emulsifier forming the L_2 area, which represented a solution with inverse micelles. This solution, the inverse middle phase (RM) (Fig. 2) containing a hexagonal array of cylinders (Fig. 1), and the lamellar neat phase (Fig. 1), denoted N in Fig. 2, showed small differences in total composition from each other and were judged suitable to demonstrate the influence of different associated structures.

The partition coefficient of dichlorvos between water and cyclohexane was determined separately using the pure compounds. This corresponds to a composition choice anywhere along the line H_2O–c-C_6H_{12} in Fig. 2. The results are given in Table I (Samples 4 and 5).

[1] Donated by Shell Co.
[2] Fluka.
[3] Eastman.
[4] Beckman.

Table I—Partition Coefficient of Dichlorvos between Different Phases and Water in the Water–Cyclohexane–Commercial Polyethylene Glycol Dodecyl Ether System

Sample	Cyclohexane	L_2	RM	N
1	—	4.7	5.9	—
2	—	4.6	5.7	—
3	—	—	5.8	5.5
4	2.2	—	—	—
5	2.2	—	—	—

The three phases H_2O, L_2, and RM could easily be separated from a total composition, denoted A in the diagram. The actual composition of the phases L_2 and RM in the three-phase region was not determined, but the fact that an increase of the cyclohexane content in the total composition A gave a sample containing only H_2O and L_2 was evidence of a very narrow three-phase region (approximately the one indicated in Fig. 2). The partition of dichlorvos between these three phases was determined in duplicate samples. The results are given in Table I (Samples 1 and 2) as partition coefficients for the two phases L_2 and RM against water.

The separation of the three phases H_2O, N, and RM was difficult, and it was considered sufficient to determine the partition between N and RM, which could easily be separated. The composition of the phase RM in equilibrium both with H_2O and N was necessarily close to its composition in equilibrium with H_2O and L_2. This is evident from an inspection of Fig. 2, in which the probable three-phase area containing H_2O, RM, and N is indicated. Regarding this similarity in composition of RM, it was judged reasonable to assume an RM–H_2O partition coefficient for the three phase H_2O, RM, and N very close to the corresponding coefficient for the three phases H_2O, RM, and L_2. The partition coefficients RM–H_2O and N–H_2O in Table I (Sample 3) were consequently calculated using an average value for the RM partition coefficient against water from Samples 1 and 2.

The partition coefficients are given in Table I. The results show that the dichlorvos actually showed a higher partition coefficient for the liquid crystalline and the L_2 phases than for the hydrocarbon phase. The difference between the two liquid crystalline phases was not significant.

The next system was constructed to answer the question of the importance of structure more generally; micelles were also taken into account. The system contained 1-octanoic acid and 1-aminooctane in a 1:1 molecular ratio. These two components form the salt octylammonium octanoate, which made the system contain a surfactant with a strong dipolar element similar to lecithin.

The water, *p*-xylene, and 1-octanoic acid–1-aminooctane (1:1) system (Fig. 3) consists of pure water, a *p*-xylene solution (L_2) containing the surfactant pair and solubilized water, and a lamellar liquid crystalline phase (N) (15). According to IR and NMR data, the *p*-xylene solution contains ion-pairs of the amphiphiles in a 1:1 ratio (16). When water is added, inverse micelles are formed simi-

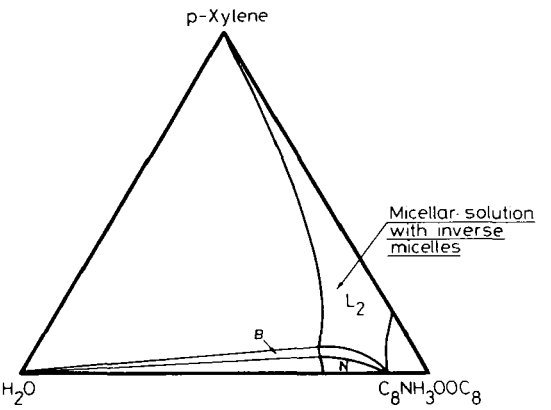


Figure 3—*Water, p-xylene, and the emulsifier octylammonium octanoate formed two liquid phases [pure water and a p-xylene solution (L_2) containing the emulsifier and solubilized water and liquid crystalline phase (N)].*

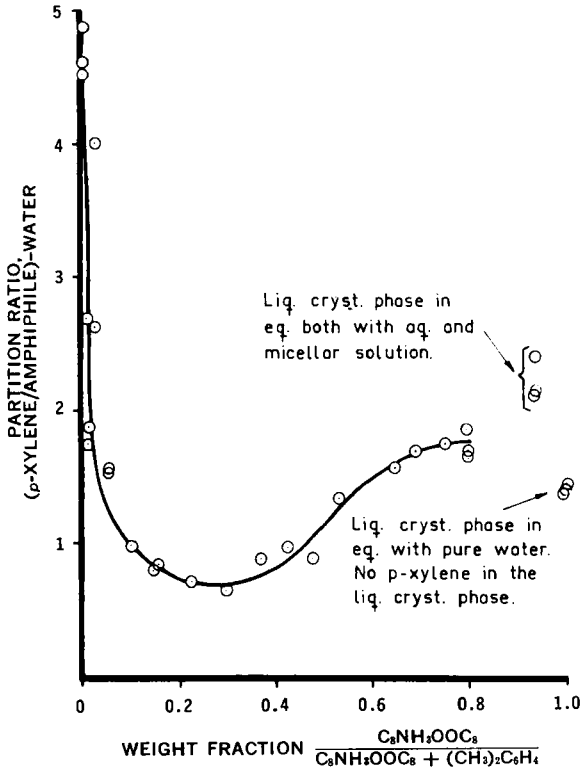


Figure 4—*Partition of dichlorvos between the phases in the water, p-xylene, and octylammonium octanoate system plotted against the weight fraction of the emulsifier.*

lar to those in the corresponding system in which the surfactant consisted of 1-aminooctane only (17).

The results (Fig. 4) show that the structure of the phases had a pronounced influence on the partition of dichlorvos between the phases. Small additions (~2%) of the two amphiphilic compounds reduced the partition coefficient to less than 50% of the initial value for *p*-xylene–water. When a weight basis fraction of added amphiphiles to *p*-xylene reached one-third, the partition coefficient reached a minimum, being 16% of the value for *p*-xylene–water. Further additions of the amphiphiles increased the coefficient. The three-phase region of water, isotropic liquid (L_2) phase with maximum amount of amphiphiles (B in Fig. 3), and liquid crystalline phase gave partition coefficients significantly higher for the liquid crystalline phase toward water (2.2) than the corresponding coefficient for the isotropic micellar solution and water (1.75).

Further reduction to zero of the *p*-xylene percentage within the liquid crystalline phase caused a reduction of the partition coefficient from 2.2 to 1.35.

DISCUSSION

The results obtained made evident that the associated colloid structures present in micellar solutions and in liquid crystalline phases had a pronounced influence on the partition of dichlorvos.

This influence was demonstrated by the drastic changes of the partition coefficient toward water when the initial addition of octylammonium octanoate to *p*-xylene was made. The initial steep reduction of the partition coefficient and its minimum at a weight fraction of about 0.3 are features that have to be explained from changes in the colloid and subcolloid associated structures. The exact determination of these structures was not within the realm of the present investigation, but a comparison with findings of earlier studies on similar systems may be of value, giving the general conditions that may be expected (17).

At low weight fractions of the salt, the salt is dissolved as separate ion-pairs with one or two water molecules attached to each pair. At higher concentrations, the addition of water gives rise to the formation of larger aggregates, the size of which determines if they are micelles. The onset of the formation of larger aggregates is seldom as sudden as in aqueous systems, where the critical micellization concentration is experienced as a narrow concentration range.

Considering these general statements, it appears probable that the minimum in the partition coefficient curve (Fig. 4) was related to the formation of larger aggregates. The increase of the partition coefficient may then be a rational consequence of the formation of colloid size volumes containing water and polar parts, which are then separated from the hydrocarbon parts of the solution (Fig. 1). The enhancement of the partition coefficient when the structure changed from the micellar solution to the liquid crystalline phase is in accordance with this opinion; the change implies an even stricter separation into polar and nonpolar colloid dimensions (Fig. 1).

The reduction of the partition coefficient when *p*-xylene was removed from the liquid crystalline phase was considerably higher than what could be expected with regard to the reduction of the nonpolar volume in the liquid crystalline phase. The reduction in nonpolar volume was less than 3%; the decrease of the partition coefficient was about 10 times that value. A continued investigation of possible changes in the localization of dichlorvos would be of interest with regard to this result.

For the system with a nonionic emulsifier, the partition coefficient between cyclohexane and water was lower than the coefficient between *p*-xylene and water. The liquid crystalline phases in the latter system, on the other hand, caused the value of the partition coefficient toward water to be higher than that between hydrocarbon and water; this finding was another example of the importance of structure conditions.

The importance of the results is not due only to the changes in partition coefficient. It is well known (18, 19) that variations in the polarity and hydrogen bonding capacity of the nonaqueous liquid generally give rise to a broad range of partition coefficient values. However, this fact is not easily applicable to ordered systems, since even the value of a liquid with the polarity and hydrogen bond capacity adjusted to average values for a liquid crystalline phase may be in error to a high degree. This is well displayed by the two systems used in the present investigation. Average properties calculated on the basis of the molecular composition are of no use for the determination of the partition coefficient when it passes through a maximum and a minimum; this was the case when the weight ratio of octylammonium octanoate to *p*-xylene was varied (Fig. 4). When these results are considered, the estimation of partition coefficients between nerve tissues and surrounding plasma from the coefficients of simple liquids appears questionable.

REFERENCES

(1) D. F. Heath, "Organophosphorus Poisons," Pergamon Press, Oxford, England, 1961.

(2) G. Schrader, "Die Entwicklung neuer insektizider Phosphorsäure-Ester," Verlag Chemie Gmbh, Weinheim, Germany, 1963.

(3) S. Franke, "Lehrbuch der Militärchemie," Band 1, Deutscher Militärverlag, Berlin, Germany, 1967, p. 297.

(4) D. Nachmansohn, *Science*, **168**, 1059(1970).

(5) F. C. G. Hoskin, P. Rosenberg, and M. Bozin, *Proc. Nat. Acad. Sci. USA*, **55**, 1231(1966).

(6) F. C. G. Hoskin, L. T. Kremzner, and P. Rosenberg, *Biochem. Pharmacol.*, **18**, 1727(1969).

(7) R. J. McIsaac and G. B. Koelle, *J. Pharmacol. Exp. Ther.*, **126**, 9(1969).

(8) F. C. G. Hoskin, *Science*, **172**, 1243(1971).

(9) J. F. Danielli and H. Davson, *J. Cell. Comp. Physiol.*, **5**, 495(1935).

(10) S. Friberg, *J. Amer. Oil Chem. Soc.*, **48**, 578(1971).

(11) V. Luzzati, in "Biological Membranes," D. Chapman, Ed., Academic Press, London, England, 1968, p. 71.

(12) H. Kavanau, "Structure and Function in Biological Membranes," Holden-Day, San Francisco, Calif., 1965.

(13) "2,2-Dichlorovinyl Dimethyl Phosphate," Bibliography, Shell Research Ltd., London, England, 1971.

(14) M. Gales, Jr., E. Julian, and R. Croner, *J. Amer. Water Works Ass.*, **58**, 1363(1966).

(15) S. Friberg and L. Rydhag, *Kolloid-Z. Polym.*, **244**,

233(1971).
(16) B. Lincoln and S. Friberg, Presented at the Fifth International Conference on Surface Active Substances.
(17) G. La Force and B. Sarthz, *J. Colloid Interface Sci.*, **37**, 254(1971).
(18) H. Meyer and B. H. Hemmi, *Biochem. Z.*, **277**, 39(1935).
(19) R. Deked, *Monatsber. Deut. Akad. Wiss. Berlin*, **4**, 225(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 17, 1974, from *The Swedish Institute for Surface Chemistry, Box 5607, S-114 86 Stockholm, Sweden, and ‡The National Defence Research Institute, Department 4, Box 416, S-172 04 Sundbyberg 4, Sweden.

Accepted for publication October 17, 1974.

* To whom inquiries should be directed.

Synthesis of Sparsomycin Analogs as Potential Antitumor Agents

R. J. DUBOIS ×, C.-C. L. LIN, and B. L. MICHEL

Abstract □ No information is available on the structural requirements for the antitumor activity of sparsomycin, an antibiotic obtained from the fermentation broth of *Streptomyces sparsogenes*. Its high *in vivo* and *in vitro* activity, novel structure, and uncommon mode of action have, therefore, suggested the synthesis of analogs. This report describes the preparation and screening of a series of *N*-substituted 3-aryl acrylamides which are closely related to sparsomycin. Three compounds exhibited some tumor inhibition but insufficient to warrant further testing.

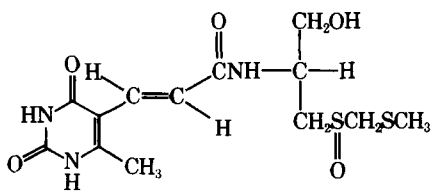
Keyphrases □ Antitumor agents, potential—synthesis and screening of sparsomycin analogs □ Cysteinol derivatives—synthesis and screening as possible anticancer agents □ 3-Aryl acrylamides, *N*-substituted—synthesis and pharmacological screening as possible anticancer agents □ Sparsomycin analogs—synthesis and screening as potential antitumor agents

The antibiotic sparsomycin (I) was first isolated in 1962 (1) from the fermentation broth of *Streptomyces sparsogenes*. Not until 1970, however, was the structure elucidation reported (2).

Following its isolation, sparsomycin was subjected to several preliminary biological tests where it displayed a broad spectrum of moderate *in vitro* activity against bacteria and moderate antifungal activity (3). Of greater interest was its very high activity against KB human epidermoid carcinoma cells (3). It also showed moderate to high inhibition in several *in vivo* tumor systems such as the Walker carcinosarcoma 256 and the sarcoma 180 solid tumor (3).

On the basis of this antitumor activity, sparsomycin was selected for Phase I clinical studies. It displayed severe eye toxicity, however, and the Phase I study was terminated (4).

Its biological activity appears to be primarily due to inhibition of protein synthesis, and this inhibition



I

Table I—NSC Numbers and Screening Data

Compound	NSC Number	Walker 256 Data, % T/C (Dose, mg/kg)
II	173109	49 (120)
III	177934	55 (40)
IV	181493	85 (50)
V	181494	64 (160)
VI	184712	96 (80)
VII	184714	107 (50)
VIII	159934	—
IX-HCl	166004	—
X	169798	—
XI	169799	—
XII	173110	—
XIII	174260	—
XIV	177936	—
XV	179898	—

has been substantiated (5, 6). Further work (7) indicated that its mechanism of action in the *Escherichia coli* system is on the 50S ribosome subunit, where it prevents peptide transfer by interfering with the function of the enzyme peptidyl transferase.

To date, no analogs of sparsomycin have been reported. Its high to moderate antitumor activity, novel structure, and somewhat uncommon mode of action have prompted the development of a structure–activity relationship by synthesis. This report describes some initial investigations.

RESULTS AND DISCUSSION

As an initial synthetic goal, the novel *N*-substituted 3-aryl acrylamides (II–VII) were chosen. These compounds represent analogs in which the uracil portion of sparsomycin has been replaced by other heterocyclic or aromatic moieties and the sulfoxide portion has been replaced by sulfur. It was believed that these compounds retained a sufficient portion of the sparsomycin structure to warrant their preparation and testing as potential antitumor agents.

Scheme I outlines the proposed synthetic approach. Condensation of the amino ester (IX) with the appropriate acrylic acid should yield esters (X–XV), which could then be selectively reduced to the desired compounds (II–VII). A synthetic pathway to the key intermediate (IX), involving the amino acid cystine, was developed (Scheme II). Since sparsomycin is optically active with a D-configuration at the asymmetric carbon atom, D-cystine would