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Crystal and Molecular Structure of Organophosphorus Insecticides. 7. Crufomate

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The crystal and molecular structure of crufomate {2-chloro-4-(1,1-dimethylethyl) methyl methylphosphoramidate, $(\text{H}_3\text{CO})(\text{H}_3\text{CNH})\text{P}(\text{O})\text{OC}_6\text{H}_3\text{Cl}(\text{CH}_3)_3$, triclinic, $P\bar{1}$, $a = 9.506$ (2), $b = 11.826$ (3), $c = 7.277$ (1) Å, $\alpha = 106.90$ (2), $\beta = 91.86$ (3), $\gamma = 105.11$ (3)°, $Z = 2$, Mo $K\alpha$ radiation} has been determined by three-dimensional x-ray analysis. The structure was solved by conventional Patterson and Fourier techniques to a final discrepancy index $R = 0.074$ for 1632 observed reflections ($F_0 > 2.5\sigma(F_0)$). The configuration is substantiated by CNDO II molecular orbital calculations and van der Waals arguments and likely features a weak electrostatic interaction which somewhat restricts rotation about the phenolic C-O bond, thus giving a probable *in vivo* model. The phosphorus-meta hydrogen distances of 5.68 and 5.13 Å fall well within the range of literature values cited for the intramolecular active site-separation distance for insect acetylcholinesterase (AChE), yet are well outside that for mammalian AChE.

The crystal structure investigation of crufomate was undertaken as a part of a study of various organophosphorus (OP) insecticides being carried on at this laboratory (Baughman and Jacobson, 1975, 1976, 1977; Gifkins and Jacobson, 1976; Rohrbaugh and Jacobson, 1976, 1977). The purpose of such a program is to better understand the relationship between structure and mechanism(s) relative to an insecticide's toxicity/activity.

In the case of acetylcholinesterase (AChE) inhibition, one would ideally like to know the three-dimensional structure of the active sites (or that of a small range of possible low-energy conformations). The complexity of this enzyme virtually prohibits direct elucidation of its structure. However, accurate structural determinations of smaller molecules such as the organophosphorus (OP) and/or carbamate insecticides, which strongly interact with the active site(s) of AChE, would allow not only inferences to be made with regards to the topography of AChE but also yield valuable molecular insight into the insecticides themselves. Such information could then lead to the construction of insecticides which would better conform structurally as well as chemically to the most favorable (minimum energy) orientation of AChE.

EXPERIMENTAL SECTION

Preparation. A sample of 99% pure crufomate was recrystallized from reagent grade carbon tetrachloride. It was necessary to evaporate the solution to dryness to obtain the colorless species.

Crystal Data. A rectangular prismatic crystal with approximate dimensions 0.30 mm \times 0.40 mm \times 0.50 mm was selected and mounted on the end of a glass fiber using Elmer's Glue-All. The crystal was then mounted on a four-circle diffractometer; three ω -oscillation photographs taken at various χ and ϕ settings indicated that the crystal was indeed single.

From these photographs 16 independent reflections were selected and their coordinates were input into an automatic

indexing program (Jacobson, 1976). The reduced cell and reduced cell scalars which resulted indicated triclinic symmetry, which was confirmed by inspection of ω -oscillation photographs taken about each of the three axes in turn. No axis showed mirror symmetry. Observed layer line spacings agreed, within experimental error, with those predicted for this cell by the indexing program.

The lattice constants were obtained from a least-squares refinement based on the precise $\pm 2\theta$ ($|\theta| > 30^\circ$) measurements of 22 strong independent reflections. At 27 °C using Mo $K\alpha$ ($\lambda = 0.70954$ Å) they are $a = 9.506$ (2), $b = 11.826$ (3), $c = 7.277$ (1) Å, $\alpha = 106.90$ (2), $\beta = 91.86$ (3), $\gamma = 105.11$ (3)°. The observed density of 1.30 ± 0.03 g cm⁻³ determined by the flotation method is in good agreement with the calculated value of 1.292 g cm⁻³ for two molecules with a molecular weight of 292.1 g mol⁻¹ in a unit cell having a volume of 750.49 Å³.

Collection and Reduction of X-Ray Intensity Data. The data were collected at 27 °C with graphite monochromated Mo $K\alpha$ radiation on an automated four-circle diffractometer designed and built in the Ames Laboratory and previously described by Rohrbaugh and Jacobson (1974). All data within a 2θ sphere of 45° $\{(\sin \theta)/\lambda = 0.539$ Å⁻¹ $\}$ in the hkl , $\bar{h}kl$, $h\bar{k}l$, and $\bar{h}\bar{k}l$ octants were measured using an ω -stepscan technique.

As a general check on electronic and crystal stability, the intensities of three standard reflections were remeasured every 75 reflections. These standard reflections were not observed to vary significantly throughout the entire period of data collection (~ 2 days). Hence, a decomposition correction was unnecessary. A total of 2993 reflections were recorded in this manner. Examination of the data did not reveal any systematic extinctions. As a Howells, Phillips, and Rogers (1950) plot indicated a centrosymmetric space group, the space group was assigned as $P\bar{1}$.

The intensity data were corrected for Lorentz and polarization effects and, since $\mu = 3.64$ cm⁻¹, absorption corrections were not made; maximum and minimum transmission factors were 0.897 and 0.834, respectively. The estimated variance in each intensity was calculated by the following equation:

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$$\sigma_1^2 = C_T + 2C_B + (0.03C_T)^2 + (0.03C_B)^2$$

where C_T and C_B represent the total and background counts, respectively, and the factor 0.03 represents an estimate of nonstatistical errors. The estimated deviations in the structure factors were calculated by the finite difference method (Lawton and Jacobson, 1968). Equivalent data were averaged and 1632 reflections with $F_o > 2.5\sigma(F_o)$ were retained for use in subsequent calculations.

SOLUTION AND REFINEMENT

The position of the chlorine atom was obtained from an analysis of a standard three-dimensional Patterson function. The remaining atoms were found by successive structure factor (Busing et al., 1962) and electron density map calculations (Hubbard et al., 1971). These atomic positions were subsequently refined by a full-matrix least-square procedure (Busing et al., 1962) minimizing the function $\sum \omega(|F_o| - |F_c|)^2$, where $\omega = 1/\sigma_F^2$. This refinement yielded a conventional discrepancy factor of $R = \sum |F_o| - |F_c| / \sum |F_o| = 0.178$. At this stage of the refinement all 18 nonhydrogen atoms had been refined using isotropic thermal parameters. The scattering factors used were those of Hanson et al. (1960) modified for the real and imaginary parts of anomalous dispersion (Templeton, 1962). The scattering factor tables for hydrogen were those of Stewart et al. (1965).

Ring hydrogen atom positions were fixed at 0.95 Å from the corresponding carbons. Analysis of an electron density difference map (Hubbard et al., 1971) did not reveal either the individual methyl or amide hydrogen positions. The methyl hydrogens were inserted in approximately tetrahedral positions of the corresponding methyl carbon and the nonhydrogen atom bonded immediately to the methyl carbon. The methyl C-H distances were set equal to 1.0 Å; all isotropic hydrogen temperature factors were set equal to 4.5 Å². As the amide hydrogen did not appear in the electron density map, conformational disordering is likely. The amide hydrogen was not included in least-squares refinements and is not shown in any of the figures.

In the final stages of refinement, analysis of the weights, ω , was performed via the requirement that $\omega \nabla^2 (\nabla \equiv ||F_o| - |F_c||)$ should be a constant function of $|F_o|$ and $(\sin \theta)/\lambda$ (Cruickshank and Pilling, 1961). As reflections at very low and very high $(\sin \theta)/\lambda$ were somewhat overweighted, all ω 's were subsequently adjusted.

Subsequent anisotropic least-squares refinement without varying the hydrogen parameters converged to $R = 0.074$. Since this procedure yielded slightly different nonhydrogen atom positions, all of the hydrogen positions were recalculated. Further refinement cycles did not significantly alter any atomic parameters and the discrepancy factor did not change.

The final positional and thermal parameters are listed in Table I. Standard deviations were calculated from the inverse matrix of the final least-squares cycle. Bond lengths and angles are listed in Table II and III, respectively (Busing et al., 1964). Dihedral angles and least-square planes are listed in Table IV.

DESCRIPTION OF STRUCTURE AND DISCUSSION

The phenoxy group in crufomate shown in Figures 1 and 2 (Johnson, 1971) is, as expected, essentially planar (cf. Table IV, plane II). In addition, the *tert*-butyl group is nearly symmetrically disposed about C(4) as shown in Table IV, plane III, and Figure 1.

Referring to Table III it is noted that angles of the type O(2)=P-O or N are all greater than the tetrahedral angle of 109.47°. Those angles about the phosphorus not in-

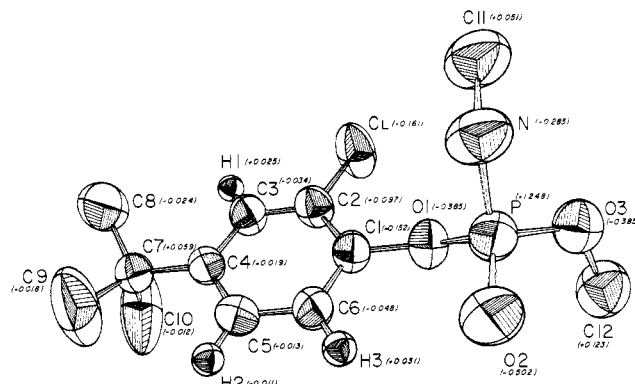


Figure 1. The crufomate molecule showing 50% probability ellipsoids; 30% for hydrogens. For clarity methyl hydrogens are not shown.

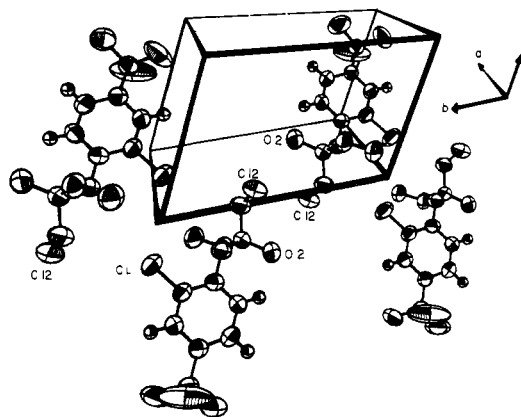


Figure 2. View of two adjacent unit cells in the *a* direction.

volving O(2) are all less than 109.47°. This was also noted with ronnel (Baughman and Jacobson, 1975), bromophos (Baughman and Jacobson, 1976), fospirate (Baughman and Jacobson, 1977), and Coroxon (Gifkins and Jacobson, 1976).

With the exception of the very weak Coulombic interactions Cl...C(12)H₃, C(10)H₃...O(3), and C(12)H₃...O(2), packing in the crufomate crystal can be regarded as primarily van der Waals in nature; only a few short intermolecular contacts are observed (cf. Tables II and III and Figure 2).

The C(1)-O(1) bond in crufomate is significantly ($\sim 5\sigma$) shorter than the methoxy C-O bond (cf. Table II), while the P-O(1) bond is $\geq 5\sigma$ longer than the P-O(3) bond. These observations, which are in agreement with CNDO II molecular orbital calculations of the Pople and Beveridge (1970) type, are consistent with a bonding formulation in which there is a weak overlap of the p_z orbital on the oxygen with the ring system which simultaneously weakens the O(1)-P bond. The latter effect should enhance phosphorylation (Clark et al., 1964). The corresponding P-O and C-O bond lengths in crufomate and Coroxon (another phosphate) are within 3σ of being identical, while only slight differences are noted in comparison with the thiophosphates.

As with ronnel, bromophos, and fospirate, the phosphorus in crufomate is on the H(3) side of a plane which is perpendicular to the ring and contains the C(1)-O(1) bond. However, the doubly bonded moiety in crufomate (O(2)) is not skewed toward the ortho chlorine as are the sulfur atoms in ronnel and bromophos.

The H(3)...O(2) and C(6)...O(2) distances of 2.46 and 3.13 Å, respectively, and the P-O(2)...H(3) and C(6)-H(3)...O(2) angles of 119.2 and 118.8°, respectively, could indicate a

Table I. Final Atomic Positional^a and Thermal^b Parameters for Crufomate

Atom	Fractional coordinates			Atomic temperature factors					
	<i>x</i>	<i>y</i>	<i>z</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Cl	0.0870 (2) ^c	0.0159 (1)	0.2456 (2)	26.8 (3)	9.3 (1)	42.7 (5)	1.2 (1)	-14.4 (3)	2.2 (1)
P	0.0547 (1)	0.3630 (1)	0.2406 (1)	14.6 (1)	9.6 (1)	23.7 (3)	2.6 (1)	0.2 (1)	5.6 (1)
O(1)	0.1487 (3)	0.2653 (3)	0.2162 (4)	18.1 (5)	10.9 (3)	22.5 (7)	4.9 (3)	-0.1 (4)	4.7 (3)
O(2)	0.1423 (4)	0.4909 (3)	0.3252 (5)	19.2 (5)	10.5 (3)	33.6 (9)	3.7 (3)	2.4 (5)	6.9 (4)
O(3)	-0.0146 (3)	0.3246 (3)	0.0256 (4)	16.9 (4)	14.9 (4)	26.8 (8)	4.1 (3)	-0.1 (5)	8.6 (4)
C(1)	0.2327 (5)	0.2582 (4)	0.3706 (6)	14.8 (6)	10.2 (4)	19.9 (9)	3.4 (4)	0.4 (6)	4.4 (5)
C(2)	0.2132 (5)	0.1449 (4)	0.3990 (6)	16.2 (6)	8.2 (4)	26 (1)	2.3 (4)	-2.8 (7)	2.4 (5)
C(3)	0.2975 (5)	0.1335 (4)	0.5470 (7)	17.0 (7)	8.4 (4)	27 (1)	3.1 (4)	-2.0 (7)	5.0 (5)
C(4)	0.4049 (4)	0.2342 (4)	0.6712 (6)	12.9 (5)	9.6 (4)	21.9 (9)	2.9 (4)	1.5 (6)	4.5 (5)
C(5)	0.4222 (5)	0.3460 (4)	0.6364 (7)	16.4 (7)	10.1 (4)	27 (1)	-0.1 (4)	-1.9 (7)	4.8 (6)
C(6)	0.3365 (5)	0.3583 (4)	0.4903 (7)	17.3 (7)	9.7 (4)	28 (1)	1.2 (4)	-3.2 (7)	7.8 (6)
C(7)	0.4997 (5)	0.2209 (4)	0.8336 (6)	13.4 (6)	11.2 (4)	25 (1)	3.3 (4)	1.1 (6)	5.7 (5)
C(8)	0.4152 (9)	0.1246 (9)	0.914 (1)	27 (1)	29 (1)	61 (3)	-4 (1)	-13 (1)	31 (1)
C(9)	0.544 (1)	0.3357 (7)	0.998 (1)	63 (3)	17 (1)	48 (2)	4 (1)	-33 (2)	7 (1)
C(10)	0.630 (1)	0.189 (1)	0.755 (1)	36 (1)	66 (3)	38 (2)	38 (2)	4 (1)	15 (2)
C(11)	-0.1815 (6)	0.2013 (5)	0.3061 (9)	19.3 (9)	13.8 (6)	35 (1)	-0.2 (6)	2.7 (9)	7.6 (8)
C(12)	0.0727 (7)	0.3438 (6)	-0.1242 (8)	22.6 (9)	18.0 (7)	25 (1)	6.7 (7)	2.4 (9)	9.5 (8)
N	-0.0793 (5)	0.3242 (3)	0.3604 (6)	17.9 (6)	10.8 (4)	32 (1)	1.1 (4)	3.6 (6)	5.9 (5)
H(1)	0.2823	0.0552	0.5645	45					
H(2)	0.4952	0.4164	0.7156	45					
H(3)	0.3496	0.4367	0.4733	45					
C8H(1)	0.3244	0.0741	0.8251	45					
C8H(2)	0.3883	0.1649	1.0435	45					
C8H(3)	0.4769	0.0703	0.9284	45					
C9H(1)	0.4781	0.3879	0.9918	45					
C9H(2)	0.5369	0.3152	1.1226	45					
C9H(3)	0.6474	0.3819	0.9947	45					
C10H(1)	0.6622	0.2332	0.6593	45					
C10H(2)	0.7111	0.2154	0.8632	45					
C10H(3)	0.6063	0.0989	0.6919	45					
C11H(1)	-0.2831	0.2076	0.3237	45					
C11H(2)	-0.1543	0.1550	0.3901	45					
C11H(3)	-0.1768	0.1568	0.1683	45					
C12H(1)	0.0879	0.4300	-0.1268	45					
C12H(2)	0.1695	0.3298	-0.0995	45					
C12H(3)	0.0214	0.2851	-0.2513	45					

^a The positional parameters for all atoms are represented in fractional unit cell coordinates. ^b The β_{ij} are defined by: $T = \exp\{-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})\}$. If only the β_{11} column is listed, this corresponds to an isotropic temperature factor. All hydrogen isotropic B's have been set equal to 4.5. Nonhydrogen thermal parameters are ($\times 10^3$); hydrogen, ($\times 10$). ^c In this and succeeding tables, estimated standard deviations are given in parentheses for the least significant figures and include the error in the lattice constants. Since the hydrogens were not refined, no standard deviations are given.

Table II. Selected Interatomic Distances (Å) for Crufomate

Bonding distances		Nonbonding distances			Total van der Waals distance (Pauling, 1960)
		Interaction	Via	Observed distance	
C(1)-C(2)	1.381 (7)	Cl ··· O(1)	Intramolecular	2.925 (4)	3.20
C(2)-C(3)	1.380 (7)	C(11)H ₃ ··· P	Intramolecular	2.700 (6)	3.9
C(3)-C(4)	1.400 (7)	C(11)H ₃ ··· O(1)	Intramolecular	3.169 (7)	3.4
C(4)-C(5)	1.387 (7)	C(11)H ₃ ··· O(3)	Intramolecular	3.090 (7)	3.4
C(5)-C(6)	1.383 (7)	C(12)H ₃ ··· P	Intramolecular	2.613 (6)	3.9
C(6)-C(1)	1.367 (7)	C(12)H ₃ ··· O(1)	Intramolecular	3.015 (6)	3.4
C(1)-O(1)	1.392 (6)	C(12)H ₃ ··· O(2)	Intramolecular	3.180 (7)	3.4
C(2)-Cl	1.727 (5)	C(8)H ₃ ··· H(1)	Intramolecular	2.605 (8)	3.2
C(3)-H(1)	0.947 (5)	C(10)H ₃ ··· H(1)	Intramolecular	3.330 (12)	3.2
C(4)-C(7)	1.531 (6)	C(9)H ₃ ··· H(2)	Intramolecular	2.587 (8)	3.2
C(5)-H(2)	0.948 (5)	C(10)H ₃ ··· H(2)	Intramolecular	3.341 (12)	3.2
C(6)-H(3)	0.948 (5)	H(3) ··· O(2)	Intramolecular	2.525 (4); 2.46 ^a	2.6
P-O(1)	1.611 (3)	P ··· H(1)	Intramolecular	5.680 (2)	(3.1)
P=O(2)	1.459 (4)	P ··· H(2)	Intramolecular	5.127 (2)	(3.1)
P-O(3)	1.566 (4)	P ··· H(3)	Intramolecular	3.006 (2)	(3.1)
P-N	1.611 (5)	C(6) ··· O(2)	Intramolecular	3.126 (7)	3.2 ^b
O(3)-C(12)	1.426 (7)	Cl ··· C(12)H ₃	Center of inversion	3.943 (7)	3.80
N-C(11)	1.456 (7)	Cl ··· Cl	Center of inversion	3.764 (3)	3.60
C(7)-C(8)	1.493 (9)	C(10)H ₃ ··· C(11)H ₃	1 cell in <i>x</i>	3.800 (12)	4.0
C(7)-C(9)	1.480 (10)	C(10)H ₃ ··· O(3)	1 cell in <i>x</i> and <i>z</i>	3.568 (12)	3.4
C(7)-C(10)	1.473 (10)	C(12)H ₃ ··· O(2)	Center + 1 cell in <i>y</i>	3.700 (7)	3.4

^a Using a C(6)-proton distance of 1.08 Å (Sutton, 1958). ^b Bond 1 (1964).

Table III. Bond Angles (Degrees) for Crufomate

C(1)-C(2)-C(3)	120.2 (4)
C(2)-C(3)-C(4)	121.9 (4)
C(3)-C(4)-C(5)	116.1 (4)
C(4)-C(5)-C(6)	122.2 (4)
C(5)-C(6)-C(1)	120.4 (4)
C(6)-C(1)-C(2)	119.2 (4)
O(1)-C(1)-C(2)	118.5 (4)
O(1)-C(1)-C(6)	122.3 (4)
Cl-C(2)-C(1)	120.2 (4)
Cl-C(2)-C(3)	119.5 (4)
H(1)-C(3)-C(2)	118.9 (5)
H(1)-C(3)-C(4)	119.1 (4)
C(7)-C(4)-C(3)	121.8 (4)
C(7)-C(4)-C(5)	122.0 (4)
H(2)-C(5)-C(4)	118.9 (5)
H(2)-C(5)-C(6)	118.9 (5)
H(3)-C(6)-C(5)	119.8 (5)
H(3)-C(6)-C(1)	119.8 (5)
C(4)-C(7)-C(8)	110.9 (4)
C(4)-C(7)-C(9)	111.5 (5)
C(4)-C(7)-C(10)	108.5 (5)
C(8)-C(7)-C(9)	105.3 (7)
C(8)-C(7)-C(10)	110.8 (9)
C(9)-C(7)-C(10)	109.8 (9)
C(1)-O(1)-P	122.8 (3)
O(1)-P-O(2)	113.7 (2)
O(2)-P-O(3)	116.4 (2)
O(2)-P-N	113.0 (2)
O(1)-P-O(3)	98.8 (2)
O(1)-P-N	108.1 (2)
O(3)-P-N	105.7 (2)
P-O(3)-C(12)	121.6 (4)
P-N-C(11)	123.4 (4)
P-O(2) ··· H(3)	94.2 (2); 119.2 ^a
P-O(2) ··· C(12) ^b	101.9 (2)
C(2)-Cl ··· C(12) ^c	143.9 (2)
P-O(3) ··· C(10) ^d	138.0 (3)
C(6)-H(3) ··· O(2) ^a	118.8

^a Using a C(6)-proton distance of 1.08 Å (Sutton, 1958).

^b Through center + one cell in y. ^c Through center.

^d One cell in x and z.

small electrostatic interaction between H(3) and O(2). Such interaction would be significantly less than those involving the amide hydrogen, but the latter does not have the opportunity to form an appreciable hydrogen bond of either the inter- or intramolecular type. In order to obtain better evidence to substantiate the possibility of a reasonably immobilized backbone in this molecule, CNDO II molecular orbital calculations (after Pople and Beveridge, 1970) were carried out for crufomate in order to obtain information on the change in the potential energy surface as a function of incremental rotations about the C(1)-O(1) or O(1)-P bonds. To accomplish the calculations with the program used though, it was necessary to "replace" the *tert*-butyl group in crufomate with a methyl function. In the case of rotation about the O(1)-P bond, the C(6)-C(1)-O(1)-P angle was kept at 53.1° (i.e., the angle in the solid state). Results of this calculation are shown in Figure 3.

Since these results were obtained using CNDO methods, the absolute values of the energies and charges, as well as the differences in energies, are not as exact as with *ab initio* calculations. The latter methods are extremely cost-prohibitive and are usually unavailable for this size of a problem. Although admittedly approximate, the energies and charges obtained in this CNDO II calculation certainly give better than "order of magnitude" information, especially since d-orbital contributions are included for the phosphorus and chlorine atoms.

Table IV. Dihedral Angles (Degrees) and Least-Squares Planes

Planes defined by:		Dihedral angles of planes ^a	
C(1)-C(3)-C(5); O(1)-O(3)-N		89.1 (2)	
P-O(1)-C(1); C(1)-C(3)-C(5)		53.1 (3)	
P-O(1)-O(2); O(1)-C(1)-P		57.1 (4)	
O(1)-C(1)-P; O(1)-O(3)-N		141.4 (3)	
C(1)-C(3)-C(5); O(1)-P-N		102.6 (2)	(away from H(3))
<hr/>			
Atom	Distance from plane, Å	Atom	Distance from plane, Å
Plane (I) ^b defined by carbons (1-6): (0.75594)X + (-0.020612)Y + (-0.62135)Z - (-0.55649) = 0			
C(1)	0.0004	C(4)	-0.0037
C(2)	0.0043	C(5)	0.0085
C(3)	-0.0026	C(6)	-0.0068
Plane (II) ^b defined by all 12 phenoxy group members: (0.76106)X + (-0.20334)Y + (-0.61599)Z - (-0.51205) = 0			
C(1)	-0.0161	C(7)	0.0130
C(2)	-0.0160	H(2)	0.0343
C(3)	-0.0159	H(3)	-0.0274
C(4)	-0.0060	C(8)	-0.7481
C(5)	0.0096	C(9)	-0.6626
C(6)	-0.0127	P	-1.0258
O(1)	0.0244	O(2)	-1.0537
Cl	0.0319	O(3)	-0.4948
H(1)	-0.0192		
Plane (III) ^b defined by O(1), C(1), C(4), C(7), C(10), and N: (0.46838)X + (0.84994)Y + (0.24128)Z - (2.90656) = 0			
O(1)	-0.0155	C(7)	-0.0083
C(1)	0.0181	C(10)	0.0036
C(4)	0.0003	N	0.0018
Plane (IV) ^b defined by P, O(1), O(3), and C(1): (0.81180)X + (0.55454)Y + (-0.18295)Z - (1.97098) = 0			
P	0.0025	O(3)	-0.0047
O(1)	0.0085	C(1)	-0.0063
Plane (V) ^b defined by P, O(2), C(6), and C(1): (-0.61217)X + (-0.07460)Y + (0.78720)Z - (0.81254) = 0			
P	0.0398	C(1)	-0.0425
O(1)	-0.5352	C(6)	0.0360
O(2)	-0.0334	H(3)	-0.2090
Plane (VI) ^b defined by N, P, O(3), and C(12): (-0.17249)X + (0.97126)Y + (0.16401)Z - (3.77236) = 0			
N	-0.0032	O(3)	0.0052
P	0.0022	C(12)	-0.0041
Plane (VII) ^b defined by C(11), N, P, and O(2): (-0.53184)X + (0.63700)Y + (-0.55802)Z - (1.23345) = 0			
C(11)	-0.0036	P	0.0026
N	0.0042	O(2)	-0.0032

^a Angles correspond to proper orientation shown in Figures 1 and 2, so that the phosphorus and O(2) are both directed toward the H(3) side of the ring. ^b Planes are defined as $c_1X + c_2Y + c_3Z - d = 0$, where X, Y, and Z are Cartesian coordinates which are related to the triclinic cell coordinates (x, y, z) by the transformations: $X = xa \sin \gamma + zc \{(\cos \beta - \cos \alpha \cos \gamma)/\sin \gamma\}$, $Y = xa \cos \gamma + yb + zc \cos \alpha$, $Z = zc \{(1 - \cos^2 \alpha - \cos^2 \beta - \cos^2 \gamma + 2 \cos \alpha \cos \beta \cos \gamma)^{1/2}/\sin \gamma\}$.

The minimum in Figure 3 coincides with a C(1)-O(1)-P-O(2) angle which is only ~9° greater than the observed angle of 57.1°. This discrepancy is not too surprising since Figure 3 represents an uncoupled degree of freedom and because of the approximate nature of the calculations. Similar results were obtained with fospirate.

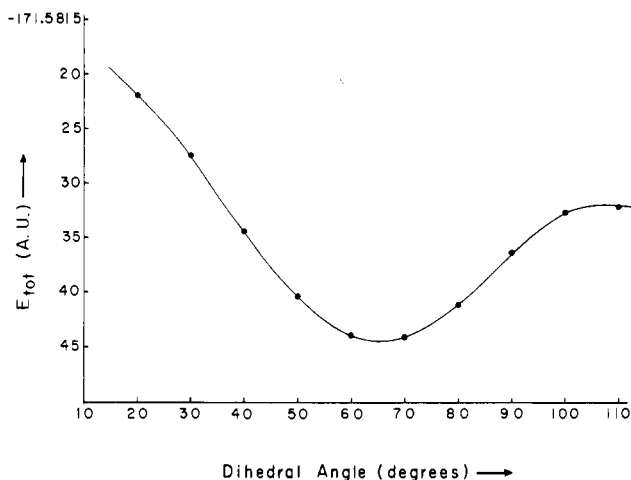


Figure 3. Total energy vs. C(1)-O(1)-P-O(2) dihedral angle from CNDO calculation.

Unfortunately, as a consequence of the approximations used in the CNDO method, the corresponding calculated potential energy curve for the rotation about the C(1)-O(1) bond is inconclusive. However, the atomic van der Waals radii of Cl and H(3) do provide a *limit* to rotation about the C(1)-O(1) bond. Looking at this rotation is most conveniently accomplished by using a three-dimensional model so that concurrent rotations about other bonds may also be performed. It turns out that the solid state structure corresponds almost exactly to the P/H(3) van der Waals limit while the P...Cl interactions is ~ 1 Å greater than the sum of their van der Waals radii. So the calculations and van der Waals restrictions seem to make the solid state structure a plausible *in vivo* model.

As discussed previously (Baughman and Jacobson, 1977), both charge density and placement are critical to inhibition. Using the CNDO II method, approximate values for the charge density distribution in crufomate can be computed, the results of which are shown in Figure 1. Examination of this figure and Table II shows that P...H(1) (5.68 Å), P...H(2) (5.13 Å), P...C(3) (4.91 Å), P...C(4) (5.29 Å), and P...C(5) (4.53 Å) are the most important in this regard. Rotation primarily about the C(1)-O(1) bond to the van der Waals limit on the Cl side of the molecule would yield the following P... $\delta(+)$ distances: P...H(1), 5.5 Å; P...H(2), 5.3 Å; P...C(3), 4.8 Å; P...C(4), 5.2 Å; P...C(5), 4.6 Å. That is, even if rotations occur, the P... $\delta(+)$ distances do not vary by more than ± 0.2 Å from the solid state distances, which should be preferred on a "time-weighted" basis.

These distances are very important since the range of active site-separation distances for insect AChE is 5.0-5.5 Å (Hollingworth et al., 1967) or 4.5-5.9 Å (O'Brien, 1963). Therefore nearly all of the pairs presented may enter into the inhibition process as reactive species. The similarities in partial charges of all of the partner atoms to phosphorus do not help to rule out any pair entirely. In addition, the distances of all but one of the five pairs fall outside of the mammalian AChE distance range of 4.3-4.7 Å (Hollingworth et al., 1967; O'Brien, 1963). On the other hand, the site-separation ranges alone essentially rule out P...C(7) (6.79 Å) and P...H(3) (3.01 Å) as primary inhibition factors for either form of AChE. On the basis of steric factors the P...meta hydrogen pair(s) may be the most plausible of the reactive moieties. This all seems to be in accord with the somewhat elevated mammalian (rat) LD₅₀ of 770-1000 mg kg⁻¹ (Eto, 1974) thus making crufomate more of a "specific" OP insecticide than, say, the nonspecific OP azinophos-methyl (Rohrbaugh and Jacobson, 1976).

The observed disparity in insect vs. mammalian LD₅₀ values typical of many OP insecticides (White-Stevens, 1971) appears to be attributable, in part, to the inherently small selective range of favorable intramolecular P... $\delta(+)$ distances (P...meta $\delta(+)$ when phenyl groups are involved). Restrictions of both the P...meta hydrogen distances and the possible steric conformations in crufomate serve to further specify any inhibition reaction route, as well as to possibly narrow the predicted active site-separation distance ranges, thus giving a better idea of the topography and possible distortions of AChE. If the molecule were not "semi-rigid", rotational freedom about the C(1)-O(1) bond would cause the P...H(1) distance to vary by $\sim \pm 0.7$ Å; P...H(2), by $\sim \pm 0.9$ Å. Parallel to this the possible conformations would have different overall steric properties.

It is noteworthy that since, as a phosphate, crufomate will not undergo *in vivo* oxidation, as the *thiophosphate* compounds can, it is likely therefore that crufomate, with the presented structure, is the predominant AChE inhibitor. Future work will include CNDO molecular orbital calculations of other OP's to obtain charge density information which can supplement x-ray crystallographic data in order to selectively design superior insecticides.

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Supplementary Material Available: A listing of the observed and calculated structure factor amplitudes (8 pages). Ordering information is given on any current masthead page.

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Crystal and Molecular Structure of Organophosphorus Insecticides. 8. Ronnel Oxon

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The crystal and molecular structure of ronnel oxon {*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphate, (H₃CO)₂P(O)OC₆H₂Cl₃, monoclinic, *P*2₁/*c*, *a* = 9.659 (5), *b* = 11.388 (2), *c* = 14.465 (9) Å, β = 130.09 (4)°, *Z* = 4, Mo Kα radiation} has been determined by three-dimensional x-ray analysis. The structure was solved by direct methods and refined by full-matrix least-squares procedures to a final discrepancy index *R* = 0.060 for 1842 observed reflections (*F*_o > 2.5σ(*F*_o)). The phosphorus-meta hydrogen distance of 5.49 Å is well within the range of literature values cited for the intramolecular site-separation distance for insect acetylcholinesterase (AChE), yet is well outside that for mammalian AChE. CNDO molecular orbital calculations are presented to show the charge density distribution in ronnel oxon.

In the case of acetylcholinesterase (AChE) inhibition, one would ideally like to know the three-dimensional structure of the active sites, or that of a small range of possible low-energy conformations. The complexity of this enzyme virtually prohibits direct elucidation of its structure. However, accurate structural determinations of smaller molecules such as the organophosphorus (OP) and/or carbamate insecticides, which strongly interact with the active site(s) of AChE, would allow not only inferences to be made with regards to the topography of AChE but also yield valuable molecular insight into the insecticides themselves. Such information could lead to the construction of insecticides which would better conform structurally as well as chemically to the most favorable (minimum energy) orientation of AChE.

The crystal structure investigation of ronnel oxon was undertaken as a part of a study of various organophosphorus (OP) insecticides being carried on at this Laboratory (Baughman and Jacobson, 1975; Gifkins and Jacobson, 1976; Rohrbaugh and Jacobson, 1977; Baughman and Jacobson, 1976; Baughman and Jacobson, 1977; Baughman et al., 1978a,b). [Structural investigations of the carbamates are concurrently being performed (Takusagawa and Jacobson, 1977a,b,c)]. The purpose of such a program is to better understand the relationship between structure and mechanism(s) relative to an insecticide's toxicity/activity. Since ronnel (*O,O*-dimethyl *O*-2,4,5-

trichlorophenyl phosphorothioate, the first reference) is a thiophosphate OP, it is logical to investigate the oxon compound in order to note any conformational similarities and/or dissimilarities which might result as a result of *in vivo* oxidation.

EXPERIMENTAL SECTION

Crystal Data. From a 99+% pure sample of the title compound, a rectangular prismatic crystal with approximate dimensions 0.22 × 0.16 × 0.18 mm was selected and mounted on the end of a glass fiber using Elmer's Glue-All. The crystal was then mounted on a four-circle diffractometer and three ω-oscillation photographs were taken at various χ and φ settings and verified that the crystal was indeed single.

From these photographs 15 independent reflections were selected and their coordinates were input into an automatic indexing program (Jacobson, 1976). The reduced cell scalars which resulted indicated monoclinic symmetry, which was confirmed by inspection of ω-oscillation photographs taken about each of the three axes in turn. Only the *b* axis showed a mirror plane. Observed layer-line spacings agreed, within experimental error, with those predicted for this cell by the indexing program.

The lattice constants were obtained from a least-squares refinement based on the precise ±2θ (45° > |2θ| > 20°) measurements of 14 strong independent reflections. At 27 °C using Mo Kα (λ = 0.70954 Å) they are *a* = 9.659 (5), *b* = 11.388 (2), *c* = 14.465 (9) Å, and β = 130.09 (4)°. The observed density of 1.63 (3) g cm⁻³ determined by the flotation method is in good agreement with the calculated value of 1.668 g cm⁻³ for four molecules with a molecular

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