Crystal and Molecular Structure of Organophosphorus Insecticides. I. Ronnel

Russell G. Baughman and Robert A. Jacobson*

The crystal and molecular structure of ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate, $(H_3CO)_2PSOC_6H_2Cl_3$, monoclinic, $P2_1/c$, a = 12.162 (9), b = 9.990 (8), and c = 11.98 (1) Å, β = 113.61 (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 = 0.051 for 1905 observed reflections ($F_o > 2.5\sigma(F_o)$). The structure displays a hydrogen–sulfur intermolecular bond in the b direction and a phosphorus which is readily accessible for phosphorylation of acetylcholinesterase. The phosphorus–"ring center" distance of 4.05 Å corresponds quite well to the 4.10-Å nitrogen–carboxyl carbon distance in acetylcholine.

Accurate three-dimensional structure information is a necessity if one is to better understand the mechanisms, steric effects, etc., involved in the biochemistry of insecticides. A series of structural investigations is currently underway at this laboratory involving a variety of insecticides (Gress and Jacobson, 1973; Gifkins and Jacobson, 1975) to obtain such structural information via X-ray diffraction techniques. Since organophosphorus insecticides are becoming increasingly important, we undertook a crystal-structure study of ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate, $(H_3CO)_2PSOC_6H_2Cl_3$), a general purpose and animal systemic insecticide.

Inactivation of the enzyme acetylcholinesterase (AChE) is generally believed to be the toxic mode of the organophosphorus (OP) insecticides and is a result of the phosphorylation of an active enzyme site. This reaction may be represented by:

$$(RO)_2POX + EH \rightarrow (RO)_2POE + HX$$

where EH = the uninhibited AChE (O'Brien, 1960). Unlike acetylcholine, the OP insecticides, or their metabolic OP derivatives/analogs, "permanently" react with (i.e. phosphorylate) the AChE (O'Brien, 1960). With a sufficiently high enough insecticide titer (~10^{-6} to 10^{-9} M), the nonphosphorylated AChE is eventually unable to hydrolyze the ACh constantly being produced during synaptic transmission. Autotoxicosis via nervous system disruption results. In mammals the nervous system disruption leads to respiratory failure and asphyxiation; the ultimate cause of the insect's expiration is unknown (O'Brien, 1960).

The phosphorylation of AChE is due, in part, to the similarities in charge distribution, size, and shape of the many OP insecticides to acetylcholine at the corresponding chemically active portions of each molecule. The phosphate ester corresponds to the acetyl group; a portion of the X group corresponds to the quaternary nitrogen (Fukuto, 1971). Using the active site environment of AChE proposed by Krupka (1964) as a model, one notes that the hydroxyl group from a serine unit is utilized in the phosphorylation process; this unit corresponds to the esteratic site of Wilson's and Bergmann's papers (Wilson et al., 1950; Bergmann and Segal, 1954; Bergmann, 1955; etc.). Concurrently an X group may interact with the anionic site on the AChE model.

Inhibition of AChE by substituted phenyl diethyl phosphates has already been shown to be a function of the Hammett σ constant (Fukuto and Metcalf, 1956). However, as noted by Fukuto (1971), Hansch and Deutsch (1966), O'Brien (1960), Fukuto and Metcalf (1956), and Canepa et al. (1966), steric effects including van der Waals interactions of the OP insecticide molecule's substituents should also be considered as playing a significant role in the degree of inhibition attained. Consequently, a three-dimensional visualization is a key to understanding the overall process.

Ames Laboratory, E.R.D.A., and Iowa State University, Ames, Iowa 50010.

EXPERIMENTAL SECTION

Preparation. A sample of 99% pure ronnel was recrystallized from reagent grade carbon tetrachloride. This solution had to be evaporated to dryness to obtain the solid colorless species.

Crystal Data. A rectangular prismatic crystal with approximate dimensions 0.4 mm × 0.2 mm × 0.1 mm was selected and housed in a 0.2 mm (i.d.) thin-walled Lindemann glass capillary with the long axis of the crystal coincident with the axis of the capillary. Preliminary oscillation photographs indicated a single crystal with 2/m (monoclinic) symmetry. The crystal was then mounted on a four-circle diffractometer and three ω-oscillation photographs were taken at various χ and ϕ settings.

From these photographs 11 independent reflections were selected and their coordinates were input into our automatic indexing program (Jacobson, 1974). The reduced cell and reduced cell scalars which resulted from this program 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.

The lattice constants were obtained from a least-squares refinement using the Nelson-Riley extrapolation function (Williams, 1964) based on the precise $\pm 2\theta$ ($|2\theta| > 20°$) measurements of 11 strong independent reflections. At 30° using Mo Kα (λ = 0.70954 Å) they are a = 12.162 (9), b = 9.990 (8), and c = 11.98 (1) Å, and β = 113.61 (4)°. The observed density of 1.62 ± 0.02 g cm^{-3} determined by the flotation method is in good agreement with the calculated value of 1.60 g cm^{-3} for four molecules per unit cell.

Collection and Reduction of X-Ray Intensity Data. The data were collected at room temperature (24°) with graphite monochromated Mo Kα X-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° ($(\sin\theta)/\lambda$ = 0.538 Å^{-1}) in the hkl and $hk\bar{l}$ octants were measured, using the step-scan technique.

As a general check on electronic and crystal stability, the intensities of three standard reflections were remeasured every 25 reflections. These standard reflections were not observed to vary significantly throughout the entire period of data collection (~2.5 days). Hence, a decomposition correction was unnecessary. A total of 3641 reflections were re-

Table I. Final Atomic Positional[a] and Thermal[b] Parameters for Ronnel

Atom	Fractional coordinates			Atomic temperature factors					
	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Cl(1)	−0.1075 (1)[c]	0.3789 (1)	0.5348 (1)	11.5 (1)	16.3 (2)	14.4 (1)	0.1 (1)	5.9 (1)	−5.1 (1)
Cl(2)	−0.2529 (1)	0.1777 (1)	0.3210 (1)	6.8 (1)	18.6 (2)	13.0 (1)	−1.2 (1)	2.9 (1)	−3.1 (1)
Cl(3)	0.1618 (1)	−0.0289 (1)	0.3372 (1)	11.5 (1)	16.3 (2)	14.7 (1)	4.1 (1)	4.8 (1)	−1.9 (1)
S	0.2977 (1)	0.3829 (1)	0.3867 (1)	10.2 (1)	18.6 (2)	12.1 (1)	4.4 (1)	4.0 (1)	7.0 (1)
P	0.3625 (1)	0.2748 (1)	0.5294 (1)	6.4 (1)	13.5 (1)	9.7 (1)	0.7 (1)	1.9 (1)	3.2 (1)
O(1)	0.2779 (2)	0.1607 (3)	0.5440 (3)	7.1 (3)	12.5 (4)	13.7 (4)	0.4 (3)	1.4 (3)	3.0 (3)
O(2)	0.4003 (6)	0.3455 (5)	0.6531 (4)	45 (1)	17.9 (7)	10.7 (5)	−14.3 (8)	1.0 (6)	−0.0 (5)
O(3)	0.4726 (4)	0.1865 (5)	0.5473 (5)	13.2 (5)	40 (1)	35.0 (9)	15.4 (6)	15.3 (6)	26.9 (9)
C(1)	0.1522 (4)	0.1673 (4)	0.4911 (4)	6.9 (4)	10.6 (6)	9.4 (5)	−0.0 (4)	1.9 (4)	2.2 (4)
C(2)	0.0917 (4)	0.2563 (5)	0.5353 (4)	8.7 (5)	11.3 (6)	8.7 (5)	−1.4 (4)	2.4 (4)	−1.1 (4)
C(3)	−0.0330 (4)	0.2604 (4)	0.4830 (4)	8.4 (5)	11.2 (6)	8.9 (5)	−1.4 (4)	3.7 (4)	−1.3 (4)
C(4)	−0.0969 (3)	0.1742 (4)	0.3888 (4)	6.7 (4)	11.6 (6)	7.9 (4)	−0.7 (4)	3.0 (3)	0.0 (4)
C(5)	−0.0360 (4)	0.0830 (5)	0.3465 (4)	9.2 (5)	11.0 (6)	7.2 (4)	−1.0 (4)	2.3 (4)	−0.9 (4)
C(6)	0.0879 (4)	0.0818 (4)	0.3961 (4)	8.5 (5)	10.1 (5)	9.4 (5)	0.9 (4)	3.7 (4)	0.9 (4)
C(7)	0.4183 (6)	0.4752 (8)	0.6864 (6)	14.6 (7)	23 (1)	16.7 (8)	3.6 (8)	4.8 (7)	−3.4 (8)
C(8)	0.5519 (6)	0.1965 (7)	0.4929 (6)	12.8 (7)	22 (1)	17.9 (8)	6.5 (7)	5.4 (7)	2.5 (7)
H(1)	0.134 (4)	0.306 (5)	0.607 (4)	22 (7)[b]					
H(2)	−0.077 (4)	0.024 (4)	0.282 (4)	12 (6)					
C(7)–H(1)	0.342961	0.527063	0.642165	45					
C(7)–H(2)	0.441307	0.482965	0.776275	45					
C(7)–H(3)	0.484285	0.512326	0.665753	45					
C(8)–H(1)	0.610363	0.270275	0.532276	45					
C(8)–H(2)	0.596347	0.110131	0.502560	45					
C(8)–H(3)	0.507334	0.216614	0.404405	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 methyl hydrogen isotropic β's have been set equal to 4.5. Nonhydrogen thermal parameters are ($\times 10^3$). All hydrogen thermal parameters are ($\times 10$). [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 methyl hydrogens were not refined, no standard deviations are given.

corded in this manner for the hkl and $hk\bar{l}$ octants. Examination of the data revealed the following systematic absences: $h0l$ when $l = 2n + 1$ and $0k0$ when $k = 2n + 1$. These absences uniquely determine the space group as $P2_1/c$.

The intensity data were corrected for Lorentz polarization effects and, since $\mu = 9.26$ cm^{-1}, absorption corrections were made. The estimated error in each intensity was calculated by:

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

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 only those reflections where $F_o > 2.5\sigma(F_o)$ were retained for use in subsequent calculations. This yielded 1905 reflections.

Solution and Refinement. The program MULTAN (Main et al., 1971) was employed to obtain the phases for the 499 strongest reflections. The resultant E map (Hubbard et al., 1971) using the best figure of merit unambiguously showed all 16 nonhydrogen positions.

These atoms were subsequently refined by a full-matrix least-squares procedure (Busing et al., 1962) minimizing the function $\Sigma\omega(|F_o| - |F_c|)^2$, where $\omega = 1/\sigma_F^2$, to a conventional discrepancy factor of $R = \Sigma||F_o| - |F_c||/|F_o| = 0.057$. At this stage all 16 nonhydrogens had anisotropic temperature factors. The scattering factors used were those of Hanson et al. (1960), modified for the real and imaginary parts of anomalous dispersion (Templeton, 1962).

An independent refinement of the ring hydrogen parameters was followed by analysis of an electron density difference map which revealed the methyl hydrogen positions. These positions were then fitted, via a least-squares technique, to a tetrahedral model using the corresponding precise oxygen and carbon positions. The C–H distances were set equal to 1.0 Å with isotropic hydrogen temperature factors set equal to 4.5 Å^2.

Subsequent least-squares refinement without varying the methyl hydrogen parameters converged to a final $R = 0.051$. Since this procedure yielded slightly different methoxy carbon and oxygen positions, the methyl hydrogen positions were recalculated. Further refinements did not significantly alter any atom parameters; the R 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 Tables II and III, respectively (Busing et al., 1964). Dihedral angles and least-squares planes are listed in Table IV.

DESCRIPTION OF STRUCTURE AND DISCUSSION

The phenoxy group in ronnel shown in Figures 1 and 2 (Johnson, 1971) is, as expected, essentially planar (cf. Table IV). The thiophosphate group is tilted toward the H(1) side of the phenoxy group while the sulfur atom is twisted away from the C(1)–O(1)–P plane toward the Cl(3) side of the ring (cf. Table IV and Figure 2).

The molecules stack through the centers of inversion (cf. Figure 2) "causing" the phenoxy groups' planes to be parallel within limits given in Table IV. Crystalline stability in the y direction can be seen to be partly a result of the hydrogen bond from the acidic H(2) to the sulfur via the twofold screw operation (cf. Table II and Figure 2).

Table II. Selected Interatomic Distances (Å) for Ronnel

Bond	Distances	Nonbonding distances	Via	Obsd distance	Total van der Waals distances (Pauling)
C(1)–C(2)	1.388 (7)				
C(2)–C(3)	1.389 (7)				
C(3)–C(4)	1.382 (6)	S···H(2)	2_1	2.98 (5)	3.05
C(4)–C(5)	1.393 (7)	P=S···H(2)	2_1	4.66 (5)	4.95[a]
C(5)–C(6)	1.379 (7)	Cl(1)···H(2)	*c* glide	2.99 (4)	3.0
C(6)–C(1)	1.384 (7)	C(5)–H(2)···Cl(1)	*c* glide	3.495 (6)	3.91[a]
		Cl(2)···C(8)–H(1)	*c* glide	3.217 (3)	3.0
C(1)–O(1)	1.400 (6)	C(8)–C(8)–H(1)···Cl(2)	*c* glide	3.886 (8)	3.8[a]
C(2)–H(1)	0.95 (5)	O(2)···C(8)–H(3)	*c* glide	2.822 (5)	2.6
C(3)–Cl(1)	1.750 (5)	O(3)···C(7)–H(2)	*c* glide	3.543 (7)	2.6
C(4)–Cl(2)	1.736 (5)				
C(5)–H(2)	0.93 (5)	C(7)–H(2)···C(8)–H(2)	*c* glide	2.76	2.4
C(6)–Cl(3)	1.745 (5)	C(7)–H(2)···C(8)–H(3)	*c* glide	2.45	2.4
		C(7)–H(3)···H(3)–C(8)	*c* glide	3.77 (1)	4.0[a]
O(1)–P	1.592 (4)				
P=S	1.903 (2)	Cl(2)···C(8)–H(2)	1 cell in *x*	3.430 (1)	3.0
P–O(2)	1.535 (5)	Cl(2)···H(3)–C(8)	1 cell in *x*	3.722 (7)	3.8[a]
P–O(3)	1.545 (4)				
O(2)–C(7)	1.347 (8)	S···C(7)–H(1)	Intramolecular	3.221 (2)	3.05
O(3)–C(8)	1.369 (7)	S···C(7)–H(3)	Intramolecular	3.442 (2)	3.05
		S···C(8)–H(3)	Intramolecular	2.978 (2)	3.05
		O(2)···C(7)–H(2)	Intramolecular	1.927 (5)	2.6
		O(2)···C(7)–H(3)	Intramolecular	1.928 (5)	2.6
		O(3)···C(8)–H(1)	Intramolecular	1.945 (5)	2.6
		O(3)···C(8)–H(2)	Intramolecular	1.946 (5)	2.6
		O(3)···C(8)–H(3)	Intramolecular	1.946 (5)	2.6

[a] Includes distance(s) from this table and assumes linear addition of radii.

Table III. Bond Angles (Degrees) for Ronnel

Angle	Degrees	Angle	Degrees
C(1)–C(2)–C(3)	119.9 (5)	H(1)–C(2)–C(3)	119 (3)
C(2)–C(3)–C(4)	120.0 (4)	H(1)–C(2)–C(1)	120 (3)
C(3)–C(4)–C(5)	120.0 (4)	C(6)–C(1)–O(1)	119.6 (4)
C(4)–C(5)–C(6)	119.9 (5)	C(2)–C(1)–O(1)	120.5 (4)
C(5)–C(6)–C(1)	120.3 (4)	C(1)–O(1)–P	123.5 (3)
C(6)–C(1)–C(2)	119.9 (4)	S–P–O(1)	117.0 (2)
Cl(3)–C(6)–C(1)	120.8 (4)	S–P–O(2)	117.4 (2)
Cl(3)–C(6)–C(5)	118.9 (4)	S–P–O(3)	118.0 (2)
H(2)–C(5)–C(6)	118 (3)	O(2)–P–O(3)	103.0 (4)
H(2)–C(5)–C(4)	122 (3)	O(2)–P–O(1)	100.2 (3)
Cl(2)–C(4)–C(5)	118.9 (4)	O(1)–P–O(3)	98.0 (3)
Cl(2)–C(4)–C(3)	121.1 (4)	P–O(2)–C(7)	132.7 (5)
Cl(1)–C(3)–C(4)	120.7 (4)	P–O(3)–C(8)	139.1 (4)
Cl(1)–C(3)–C(2)	119.2 (4)		

The unusually elongated methoxy oxygen ellipsoids (cf. Figure 1) suggest some disordering of these atoms. Therefore, an attempt was made to account for such disorder via a refinement using two half-oxygens for each methoxy oxygen approximately displaced by 0.9 Å along the major axis of the ellipsoid. No splitting of methyl carbons was attempted though due to the much smaller elongation of these ellipsoids. This refinement reduced R by only 0.010 and did not produce a physically meaningful result. More importantly, distances and angles in the remaining and chemically significant part of the molecule remained essentially unchanged throughout either refinement. Therefore, only the time-averaged model will be reported here.

Dilation of the P–O(2)–C(7) and P–O(3)–C(8) angles (cf. Table III) can easily be explained as a result of intramolecular van der Waals repulsions of the sulfur and methyl hydrogens. In fact, many of the hydrogens are less than the calculated van der Waals distance from the sulfur (cf. Table II).

The S–P–O set of angles ranges from 117 to 118°; the O–P–O set ranges from 98 to 103° (cf. Table III). Thus, the phosphate group of ronnel displays a distorted tetrahedral geometry similar to H_3PO_4 (Furberg, 1955) and coroxon (Gifkins and Jacobson, 1975). The distorted geometry effectively corresponds to a tetrahedron which has been elongated along the threefold axis, forming a trigonal pyramid.

In the present example there is rotational hindrance of the ester about the C(1)–O(1) bond arising from S···H(1) and S···Cl(3) interactions (cf. Figure 1). Each methyl group's rotation about a P–O(2 or 3) bond is restricted due to methyl hydrogen–sulfur interactions vs. lone pair–lone pair repulsions. In addition, the relatively long packing distances imply no appreciable change in the solid state vs. in vivo structures.

The nitrogen to carboxyl carbon distance in the acetylcholine bromide salt is 4.10 Å (Canepa et al., 1966). Since this is a *direct* measurement, as opposed to a deduced measurement, it is probably a better value for comparative analyses, as it is frequently possible to make solid state and significant in vivo comparisons.

Using Krupka's (1964) model and the distance of ~4.10 Å as guidelines, the following distances become quite interesting: P to H(2), 5.51; P to H(1), 2.83; S to H(1), 3.95; S to H(2), 5.49 Å. These distances would seem to rule out the utilization of the acid ring hydrogens as an electrophilic "anchor" to the anionic site of AChE. However, the distance from the phosphorus to the center of the $\delta+$ ring is 4.05 Å. If only a weak bond is required for the anchoring of the nonesteratic portion of the molecule, the ring–anionic

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

Planes defined by	Dihedral angle of planes[a]
C(1)–C(3)–C(5); C(1)–O(1)–P	69.4 (4) (toward H(1))
C(2)–C(4)–C(6); C(1)–O(1)–P	70.3 (3) (toward H(1))
C(1)–O(1)–P; O(1)–P–S	26.6 (4) (toward Cl(3))
C(1)–C(3)–C(5); O(1)–O(2)–O(3)	23.5 (2)

Plane[b] defined by carbons 1–6: $(0.31008)X + (0.68053)Y - (0.66388)Z - (-2.59893) = 0$

Atom	Distance from plane, Å
C(1)	0.0013
C(2)	–0.0094
C(3)	0.0059
C(4)	0.0056
C(5)	–0.0137
C(6)	0.0102

Plane[b] defined by all 12 phenoxy group members: $(0.32061)X + (-0.68689)Y + (0.65222)Z - (-2.55113) = 0$

Atom	Distance from plane, Å	Atom	Distance from plane, Å
C(1)	0.0217	Cl(1)	0.0803
C(2)	0.0123	Cl(2)	–0.0085
C(3)	0.0078	Cl(3)	0.0507
C(4)	–0.0134	H(1)	–0.0994
C(5)	–0.0340	H(2)	–0.0330
C(6)	0.0096	O(1)	0.0059

Plane[b] defined by C(1), O(1), P, and S: $(0.40141)x + (0.68891)y + (0.60355)z - (4.53683) = 0$

Atom	Distance from plane, Å
C(1)	0.0713
O(1)	–0.1355
P	0.1120
S	–0.0478

[a] Angles correspond to proper orientation shown in Figures 1 and 2, so that the phosphorus is tilted toward the H(1) side of the ring and the sulfur is directed away from H(1). This was also verified on the basis of intramolecular distances. [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: $X = xa \sin\gamma + zc((\cos\beta - \cos\alpha\cos\gamma)/\sin\gamma) = xa + zc\cos\beta$; $Y = xa\cos\gamma + yb + zc\cos\alpha = yb$, and $Z = zc\{1 - \cos^2\alpha - \cos^2\beta - \cos^2\gamma + 2\cos\alpha\cos\beta\cos\gamma)^{1/2}/\sin\gamma\} = zc\sin\beta$.

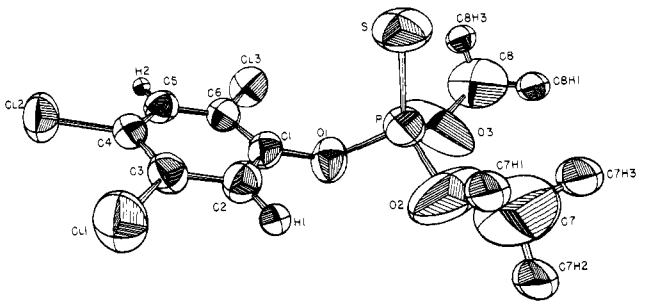

Figure 1. *The ronnel molecule showing 50% probability ellipsoids; 30% for methyl hydrogens.*

site interaction may be quite plausible. This would imply that the plane of the ring would almost have to arrive parallel to the enzyme surface. The phosphorus ester displays a "scorpion-like" configuration (Figure 1) with the C(1)–O(1)–P plane being only 20° away from the normal to the plane of the phenoxy group while the O(1)–P–S plane is only 27° from the C(1)–O(1)–P plane (cf. Table IV). This nearly perpendicular alignment of planes, then, would facilitate phosphorylation, as would the exposed phosphorus. The exposed, scorpion-like configuration has been observed in other OP insecticides (Gifkins and Jacobson, 1975; plus others still under investigation at this laboratory), regardless of whether the insecticide, or its toxic metabolic derivative, is a phosphate or thiophosphate ester. In frequent instances the study of only the parent insecticide may be all that is necessary to correlate structure with toxicity. But in other cases concurrent structural studies of the metabolic derivative(s) would also be desirable.

Work on acetylcholine bromide (Canepa et al., 1966), choline chloride (Senko and Templeton, 1960), and muscarine iodide (Jellinek, 1957) shows, as pointed out by Canepa, that the structurally similar portions of the three molecules have very nearly identical three-dimensional

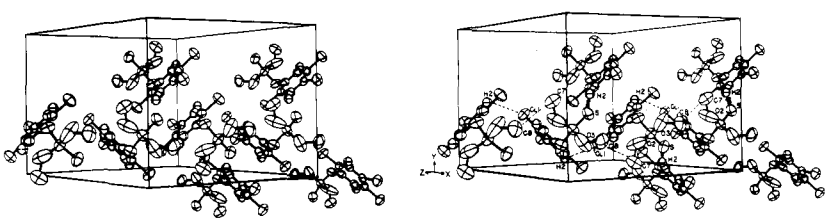

Figure 2. Stereographic view of two adjacent unit cells of ronnel illustrating intermolecular interactions.

structures. Their pharmacological effects are quite different even though the molecules are presumably unchanged in vivo vs. the solid state (Canepa et al., 1966). This implies that the portions which are not chemically and structurally in common are causing the observed variations in the inhibition of AChE. Similarly, there is a strong case for steric involvement in the mechanism of many, if not all, OP insecticides. Consequently, the precise distances afforded by X-ray crystallographic techniques will prove indispensable in the overall study of these processes.

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Supplementary Material Available. A listing of the observed and calculated structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-811.

LITERATURE CITED

Bergmann, F., *Discuss. Faraday Soc.* **20,** 126 (1955).
Bergmann, F., Segal, R., *Biochem. J.* **58,** 692 (1954).
Busing, W. R., Martin, K. O., Levy, H. A., "OR FLS, A Fortran Crystallographic Least Squares Program", U.S. Atomic Energy Commission Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962.
Busing, W. R., Martin, K. O., Levy, H. A., "OR FEE, A Fortran Crystallographic Function and Error Program", U.S. Atomic Energy Commission Report ORNL-TM-306, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1964.
Canepa, F. G., Pauling, P., Sörum, H., *Nature (London)* **210,** 907 (1966).
Fukuto, T. R., *Bull. W.H.O.* **44,** 31 (1971).
Fukuto, T. R., Metcalf, R. L., *J. Agric. Food Chem.* **4,** 930 (1956).
Furberg, S., *Acta Chem. Scand.* **9,** 1557 (1955).
Gifkins, M. R., Jacobson, R. A., manuscript in preparation, 1975.
Gress, M. E., Jacobson, R. A., *Cryst. Struct. Commun.* **3,** 485 (1973).
Hansch, C., Deutsch, E. W., *Biochim. Biophys. Acta* **126,** 117 (1966).
Hanson, H. P., Herman, F., Lea, J. D., Skillman, S., *Acta Crystallogr.* **17,** 1040 (1960).
Hubbard, C. A., Quicksall, C. O., Jacobson, R. A., "The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFPROJ, ALFFT, and FRIEDEL", U.S. Atomic Energy Commission Report IS-2625, Iowa State University and Institute for Atomic Research, Ames, Iowa, 1971.
Jacobson, R. A., "An Algorithm for Automatic Indexing and Bravais Lattice Selection. The Programs BLIND and ALICE", U.S. Atomic Energy Commission Report IS-3469, Iowa State University and Institute for Atomic Research, Ames, Iowa, 1974.
Jellinek, F., *Acta Crystallogr.* **10,** 277 (1957).
Johnson, C. A., "OR TEP-II: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations", U.S. Atomic Energy Commission Report ORNL-3794 (Second Revision with Supplemental Instructions), Oak Ridge National Laboratory, Oak Ridge, Tenn., 1971.
Krupka, R. M., *Can. J. Biochem.* **42,** 677 (1964).
Lawton, S. L., Jacobson, R. A., *Inorg. Chem.* **7,** 2124 (1968).
Main, P. M., Woolfson, M. M., Germain, G., "MULTAN: A Computer Program for the Automatic Determination of Crystal Structures", Department of Physics, University of York, York, England, 1971.
O'Brien, R. D., "Toxic Phosphorus Esters", Academic Press, New York, N.Y., 1960.
Rohrbaugh, W. J., Jacobson, R. A., *Inorg. Chem.* **13**(11), 2535 (1974).
Senko, M. E., Templeton, D. H., *Acta Crystallogr.* **13,** 281 (1960).
Templeton, D. H., in "International Tables for X-Ray Crystallography", Vol. III, The Knoch Press, Birmingham, England, 1962, Table 3.3.2c, pp 215–216.
Williams, D. E., "LCR-2, A Fortran Lattice Constant Refinement Program", U.S. Atomic Energy Commission Report IS-1052, Iowa State University and Institute for Atomic Research, Ames, Iowa, 1964.
Wilson, I. B., Bergmann, F., Nachmanson, D., *J. Biol. Chem.* **186,** 781 (1950).

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An Improved Automated Determination of Riboflavine in Food Products

David C. Egberg* and Richard H. Potter

A continuous flow scheme employing in-line permanganate oxidative cleanup has been developed in conjunction with a rapid sample preparation procedure for the automated determination of riboflavine in food products. It was shown that permanganate oxidation is required for accurate results. The automated procedure was compared with an accepted manual method for 61 different food products showing a correlation coefficient of 0.9869 and an overall standard error between methods of 0.23 mg/100 g (10.8% relative). The automated method showed a pooled relative standard deviation of 3.3% between duplicate preparation with riboflavine levels ranging from 0.05 to 43.6 mg/100 g. It was shown that an internal standard was not required in the automated method by obtaining recovery values with each sample and observing an average recovery of 100.7 ± 3.1%. The study demonstrated that the automated procedure allows rapid sample analysis without sacrificing accuracy on the majority of food products studied.

With the increased demand for nutrient analysis in food products, there exists a definite need for faster methods of analysis. Continuous flow automation is one approach to this problem.

General Mills, Inc., James Ford Bell Technical Center, Minneapolis, Minnesota 55427.

A semiautomated method for riboflavine in food products has been reported (Technicon Instrument Co., 1972); however, the method neglects permanganate treatment for the elimination of interfering fluorescent material and offers no justification for use of an external standard. The use of an internal standard has been recommended due to the possible effects of a complex matrix, such as a food hy-