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Structure of Trofosfamide

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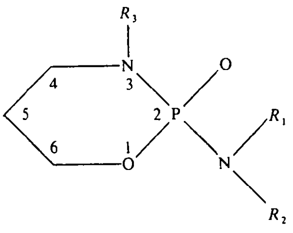
Abstract. $C_9H_{18}Cl_3N_2O_2P$, monoclinic, $P2_1/c$, $a = 13{\cdot}722\,(3)$, $b = 13{\cdot}929\,(2)$, $c = 8{\cdot}0212\,(13)$ Å, $\beta = 100{\cdot}49\,(1)°$, $Z = 4$, $D_x = 1{\cdot}426$ Mg m^{-3}, $T = 268$ K; $R = 0{\cdot}053$ for 2413 observed reflections. The ring is in the chair conformation with the phosphoryl O axial and, the bis(chloroethylamine) group equatorial.

Introduction. Cyclophosphamide (CP) and its congeners ifosfamide (IP) and trofosfamide (TP) are effective antitumor agents in clinical and experimental use. The first step in the metabolic activation of CP and its analogs is C(4)-hydroxylation by the mixed-function oxidases of liver microsomes (Arnold, Bourseaux & Brock, 1958; Brock & Hohorst, 1963; Cohen & Jao, 1970). The resulting 4-hydroxy derivative then decomposes to yield acrolein and phosphoramide mustard with the latter likely the ultimate alkylating metabolite (Colvin, Padgett & Fenselau, 1973; Connors, Cox, Farmer, Foster & Jarman, 1974; Struck, Kirk, Witt & Laster, 1975). As part of our study of the molecular structures of CP analogs and derivatives we report here the structure of TP. A preliminary report of this work was presented at the *Symposium on the Metabolism and Mechanism of Action of Cyclophosphamide* (Camerman, Smith & Camerman, 1976).



CP: $R_1 = R_2 = CH_2CH_2Cl$, $R_3 = H$
IP: $R_1 = R_3 = CH_2CH_2Cl$, $R_2 = H$
TP: $R_1 = R_2 = R_3 = CH_2CH_2Cl$

Trofosfamide was recrystallized from an ether–water mixture. The clear crystals turned yellow and then reddish-brown on exposure to X-rays. In order to retard the apparent decomposition, the crystal was immersed in a 25 mm diameter stream of very dry, cool (268 K) air during data collection. Systematic absences $h0l$, $l \neq 2n$ and $0k0$, $k \neq 2n$ confirm the space group $P2_1/c$ (C^5_{2h}, No. 14). Unit-cell parameters were determined by least-squares refinement from the 2θ, χ, φ angles of 18 reflections in the 2θ range from 31 to 48°.

Intensity data were collected on an automated four-circle diffractometer (Nb-filtered Mo radiation, $\lambda = 0{\cdot}71069$ Å), and all independent $h\bar{k}l$ and $h\bar{k}\bar{l}$ reflections having $2\theta < 50°$ (corresponding to a minimum interplanar spacing of 0·84 Å) were measured using a crystal of dimensions 0·87 × 0·73 × 0·75 mm. The θ–2θ scan method was employed with stationary 10 s background measurements taken on both sides of each reflection scan. Reflection intensities, I, were computed as $P - B(t_p/t_b)$ and the standard deviation $(I) = [P - B(t_p/t_b)^2 + (0{\cdot}03I)^2]^{1/2}$ where P and B are the total counts accumulated during the scan period, t_p, and the total background counting time, t_b, respectively. Three standard reflections ($1,\overline{11},0$, $1\bar{1}9$, $\overline{10},1,\bar{1}$) measured every 200 reflections showed a steady decline in intensity to an average minimum intensity 87% of their initial value. A quadratic decomposition curve, fit by least squares to a plot of monitor reflection intensity *versus* reflection number, was used to calculate scale factors as a function of the serial order of data collection. 26 medium to strong intensities were recollected at a lower X-ray tube current to obtain a measure of τ, the correction for saturation of the counter circuit at very high counting rates (Sletten, Sletten & Jensen, 1969). This correction was applied to the complete data set.

The intensities of 2413 unique reflections (out of a total of 2662 in the range recorded) were greater than twice their standard deviations. These reflections were classified as observed and used in the subsequent structure refinement. The standard geometrical corrections were applied to the intensities, but no corrections were made for absorption ($\mu = 0{\cdot}71$ mm^{-1}) or anomalous dispersion.

The structure was solved using the multiple-solution tangent-formula program *MULTAN* (Germain, Main

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& Woolfson, 1971), with 200 normalized structure factors with $|E| > 1{\cdot}69$. The phase of one reflection (080) was determined by the $\sum_1$ relationship at the greater than 95% probability level. The three origin-specifying reflections and two additional reflections were automatically selected as the starting set; with these reflections as input, for sets of phases for the data were derived from the Sayre relationship. The E map, calculated from the phase set with the highest figure of merit (1·18) and the lowest residual (18·2%), revealed 16 of the 17 non-hydrogen atoms in the molecule. A Fourier map based on these positions located C(13), the remaining carbon atom. With these atoms in the model, the discrepancy factor, $R = \sum||F_o| - |F_c||/\sum|F_o|$, was 0·39.

The atomic positional and anisotropic thermal parameters were refined by full-matrix least squares, where the function minimized was $\sum w(|F_o| - |F_c|)^2$. Unit weights were initially chosen, but for final refinement statistical weights, $w = 1/\sigma_F^2$, were used. Atomic scattering factors for H (Stewart, Davidson & Simpson, 1965) and for other atoms (Cromer & Mann, 1968) were taken from the literature. Computations were performed with the XRAY system (Stewart, 1976). Several cycles of refinement followed by computation of a difference Fourier map enabled coordinates to be assigned to all H atoms, except one on C(13), which was placed in a calculated position. The final cycle of least-squares refinement, which included positional and isotropic thermal parameters for all H atoms, resulted in a discrepancy factor R = 0·053 for the observed data, and 0·059 for all measured reflections. The average parameter shift to error ratio was 0·2, while the largest, 0·9, occurred in a H position. A final difference map showed two small peaks, which appeared to be alternate positions for the C(15) and C(16) atoms and may be related to the nature of the crystal decomposition. The atomic fractional coordinates and thermal parameters are given in Table 1.*

Table 1. *Final fractional coordinates of TP* ($\times 10^5$ *for* P *and* Cl; $\times 10^4$ *for* O, N *and* C) *and equivalent isotropic thermal parameters* ($\times 10^4$), *with e.s.d.'s in parentheses*

$$U_{\rm eq} = (\tfrac{1}{6}\pi^2) \textstyle\sum_i \sum_j b_{ij}\, \mathbf{a}_i\, \mathbf{a}_j.$$

	x	y	z	$U_{\rm eq}$ (Å^2)
O(1)	3195 (2)	1764 (1)	5442 (2)	523
P(2)	31238 (5)	7428 (5)	45542 (8)	419
N(3)	3194 (2)	4 (2)	6189 (3)	498
C(4)	3841 (3)	222 (2)	7831 (4)	560
C(5)	3721 (3)	1251 (2)	8338 (4)	576
C(6)	3910 (3)	1939 (2)	6992 (4)	554
O(7)	3804 (2)	602 (2)	3357 (3)	602
N(8)	1975 (2)	681 (2)	3551 (3)	438
C(9)	1729 (3)	620 (2)	1696 (4)	513
C(10)	1544 (3)	−404 (3)	1127 (4)	609
Cl(11)	12217 (8)	−4459 (9)	−11449 (10)	799
C(12)	1138 (2)	795 (2)	4451 (4)	485
C(13)	796 (3)	1815 (3)	4405 (6)	745
Cl(14)	−2872 (8)	19302 (9)	53392 (16)	946
C(15)	2983 (2)	−1021 (2)	5871 (4)	525
C(16)	3871 (3)	−1528 (3)	5489 (6)	734
Cl(17)	36020 (8)	−28098 (6)	52919 (13)	806

Discussion. The three-dimensional molecular structure of TP is shown in Fig. 1. The six-membered ring is in the chair conformation and the configuration at the P is phosphoryl O axial and the bis(chloroethylamine) group equatorial. Comparison of the conformation of the chloroethyl alkylating groups of the present molecule with those found in 4-ketocyclophosphamide (Camerman & Camerman, 1973), cyclophosphamide (García-Blanco & Perales, 1972; Karle, Karle, Egan, Zon & Brandt, 1977; Adamiak, Saenger, Kinas & Stec, 1977) and 4-hydroperoxycyclophosphamide (Camerman, Smith & Camerman, 1977) indicates that the exocyclic mustard groups, in particular, are very flexible and do not assume one favored conformation. It is likely that the important steric requirement for the chloroethyl chains is the ability for their reactive ends to be far enough apart to be able to alkylate separate bases on the DNA helices.

Bond lengths and heavy-atom angles for TP are shown in Figs. 2 and 3. These distances and angles are in good agreement with the corresponding distances and angles in cyclophosphamide and hydroperoxycyclophosphamide. However, the C—Cl bond in the chloroethyl chain attached to the ring N is somewhat longer, 1·825 (4) Å, than the C—Cl bonds in the bis(chloroethylamine) C Cl, which have an average length of 1·793 (4) Å. All of these distances are slightly greater than the expected value for the paraffinic C—Cl bond of 1·767 Å. This may indicate, particularly for the C(16)—Cl(17) bond, a partial polarization, which is consistent with the fact that this compound is an

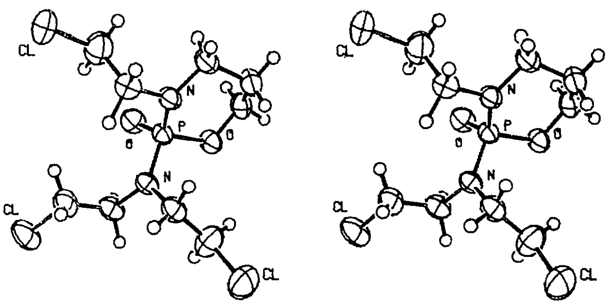

Fig. 1. Stereoscopic drawing of trofosfamide with 50% thermal ellipsoids. C and H atoms are not labeled.

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* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35627 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

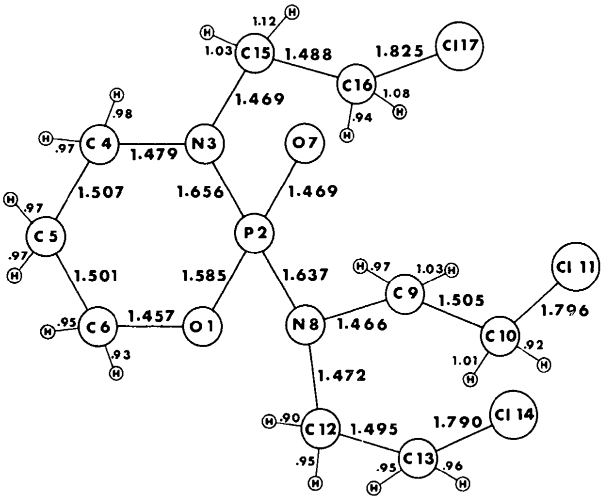

Fig. 2. Bond lengths (Å) in TP. Estimated standard deviations are 0·002–0·005 Å for 'heavy-atom' bonds and 0·06 Å for bonds involving H atoms.

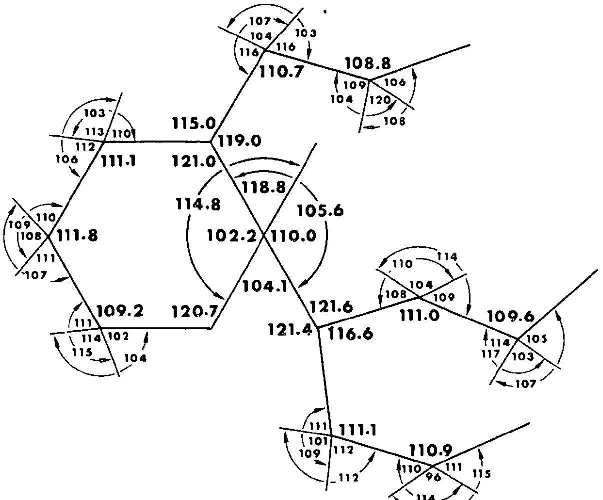

Fig. 3. Bond angles (°) in TP. Estimated standard deviations are 0·1–0·3° for 'heavy-atom' angles and 3° for angles involving H atoms.

alkylating agent. Similar long C—Cl distances were observed in the cyclophosphamide structure (Karle *et al.*, 1977; Adamiak *et al.*, 1977).

The packing diagram for TP is shown in Fig. 4. TP has no H atoms available for hydrogen bonding and shows no unusually close contacts.

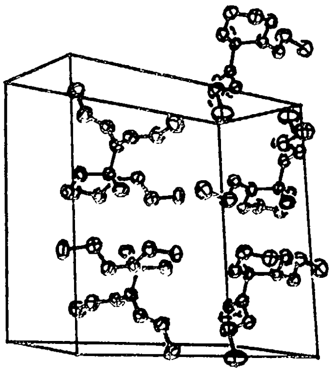

Fig. 4. The unit cell of TP. The origin is at the lower right-hand corner with the *b* axis horizontal, the *a* axis vertical, and the *c* axis pointing out of the plane of the paper.

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