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The Crystal and Molecular Structure of Ifosfamide {3-(2-Chloroethyl)-2-[(2-chloroethyl)amino]perhydro-2*H*-1,3,2-oxazaphosphorine Oxide, $C_7H_{15}Cl_2N_2O_2P$}

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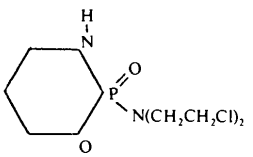
The crystal and molecular structure of ifosfamide has been determined by single-crystal X-ray diffraction methods. The crystals are orthorhombic, *Pbca*, with unit-cell dimensions: $a = 13{\cdot}358\ (2)$, $b = 21{\cdot}291\ (4)$, and $c = 8{\cdot}319\ (1)$ Å; $Z = 8$. The structure was solved by direct methods and successive Fourier synthesis. The positions of all atoms, excluding H, have been refined with anisotropic temperature factors for C, N, O, P and Cl atoms. The final R value is 0·069 for 1273 observed reflexions collected on an automatic four-circle diffractometer with Mo $K\alpha$ radiation. The molecules are held together in the crystal lattice by N—H···O hydrogen bonds. This crystal structure is compared with two other mustard-gas derivatives.

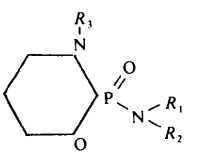
Introduction

In recent years a considerable amount of work has been devoted to the study of chemotherapy of cancer with agents related to mustard gas. Nitrogen mustard is considered as an alkylating agent. Analogous compounds produce a wide range of biological effects, such as induction of mutations (Auerbach & Robson, 1964), interference with mitosis and chromosome breakage (Darlington & Koller, 1947). All these effects are of central interest in the reactions of alkylating agents with deoxyribonucleic acid.

The purpose of the present work is to extend the structural investigation of cyclophosphamide derivatives (antitumour agents) to the specific case of ifosfamide. Ifosfamide is a new citostatic which differs from endoxan (García-Blanco & Perales, 1972) in that its two β-chloroethyl chains are not linked to the same N atoms; instead one is situated at the exocyclic N and the other at the ring N atom, which yields the structure of a secondary amide. The schematic formulae for the three compounds used in this study are given below.



Cyclophosphamide (endoxan)



$R_1 = H$
$R_2 = R_3 = (CH_2CH_2Cl)$
Ifosfamide
$R_1 = R_2 = R_3 = (CH_2CH_2Cl)$
Trofosfamide

A preliminary account of this work has been previously reported by Brassfield, Jacobson & Verkade (1975). The following paper describes the structure of trofosfamide.

Experimental

The materials for the present study were kindly provided by Asta-Werke Laboratories, Westfallen (W. Germany). Crystals of ifosfamide were obtained from a distilled solution of ether extraction in a Soxhlet apparatus, and subsequent cooling of the solution to a temperature of −10°C. Preliminary precession and Weissenberg photographs showed orthorhombic symmetry with systematically absent reflexions $hk0$ if h is odd, $0kl$ if k is odd and $h0l$ if l is odd, the space group being *Pbca*. Crystal data are given in Table 1.

The intensities were collected on a Philips PW 1100 four-circle diffractometer operating in the $\omega/2\theta$ scan mode to a limit of $2\theta = 30°$; 2082 independent reflexions were measured of which 1273 had intensities

Table 1. *Crystal data*

Lattice constants were determined by centring 25 reflexions and subsequent least-squares refinement.

Molecular formula	$C_7H_{15}N_2O_2PCl_2$
Molecular weight	261·089
Orthorhombic	$Z = 8$
Space group *Pbca*	$D_c = 1{\cdot}465$ g cm^{-3}
$a = 13{\cdot}358 \pm 0{\cdot}002$ Å	$F(000) = 1088$
$b = 21{\cdot}291 \pm 0{\cdot}004$	$\mu = 5{\cdot}255$ cm^{-1}
$c = 8{\cdot}319 \pm 0{\cdot}001$	Crystal size:
$V = 2365{\cdot}9667$ Å^3	0·40 × 0·30 × 0·28 mm
	λ(Mo $K\alpha$) = 0·7107 Å

greater than 2σ, where $\sigma^2(I) = C_p + C_b + (0{\cdot}04)^2 I^2$; C_p and C_b are peak and background counts respectively. Reflexions with intensities less than 2σ were classed as unobserved and omitted from the refinement. Graphite-monochromatized Mo $K\alpha$ radiation was used. The data were corrected for Lorentz and polarization effects but not for absorption. An overall temperature factor ($B = 4{\cdot}404$ Å^2) and scale factor were calculated from a Wilson plot and used to compute normalized structure factors (E) (Karle & Hauptman, 1956). The statistics of the E's confirmed a centrosymmetric structure.

Structure determination and refinement

The structure was solved by the multisolution tangent formula *MULTAN* (Main, Woolfson, Lessinger, Germain & Declercq, 1974). Phases of 200 reflexions with $E > 1{\cdot}6$ were used. The solution of highest figure of merit yielded an E map containing peaks corresponding to eight of the expected fourteen non-hydrogen atoms and those remaining were derived from two three-dimensional difference syntheses.

The structure was refined by a full-matrix least-squares method with anisotropic temperature factors. At this stage, H atoms appeared in a further difference synthesis, and they were subsequently verified with positions calculated from molecular geometry assuming a C–H bond of 1·00 Å and a H–C–H angle of 100·00°. The isotropic thermal parameters used were the same as those of the atoms to which the H's are bonded (Hamilton, 1959). No refinement of these parameters was performed. The scattering factors for all atoms were from *International Tables for X-ray Crystallography* (1974). Anomalous dispersion corrections were applied; the values of $\Delta f'$ and $\Delta f''$ for both Cl and P are 0·1 and 0·2, and were taken from *International Tables for X-ray Crystallography* (1974). The final R value is 0·069.

Table 2. *Positional* ($\times 10^4$) *parameters for non-hydrogen atoms*

Estimated standard deviations are given in parentheses.

	x	y	z
Cl(1)	1624 (2)	79 (1)	−1329 (3)
Cl(2)	−829 (2)	820 (1)	6306 (3)
P	1549 (1)	1983 (1)	2775 (2)
O(1)	893 (4)	2508 (2)	3260 (6)
O(2)	2671 (4)	2184 (2)	2451 (6)
N(1)	1153 (4)	1676 (3)	1097 (7)
N(2)	1681 (4)	1394 (3)	4047 (7)
C(1)	1019 (6)	652 (4)	−82 (11)
C(2)	1709 (6)	1179 (4)	275 (9)
C(3)	797 (6)	1006 (4)	4473 (9)
C(4)	166 (7)	1354 (5)	5616 (11)
C(5)	2455 (6)	1427 (4)	5308 (8)
C(6)	3459 (6)	1632 (4)	4637 (9)
C(7)	3375 (6)	2254 (3)	3767 (9)

Table 3. *Positional and thermal parameters* ($\times 10^3$) *and bond distances* (Å) *for the hydrogen atoms*

	x	y	z	U	C–H
H(1)	34	81	−57	90	1·05
H(2)	71	45	94	90	1·04
H(3)	202	133	−71	86	0·97
H(4)	225	104	91	86	0·95
H(5)	35	90	350	91	1·03
H(6)	79	56	536	91	1·20
H(7)	45	150	671	100	1·03
H(8)	−28	169	503	100	1·05
H(9)	218	170	617	68	0·99
H(10)	251	100	586	68	1·02
H(11)	398	170	540	82	0·96
H(12)	374	133	386	82	0·98
H(13)	409	234	332	82	1·04
H(14)	321	262	452	82	1·02
H(15)	85	197	49	67	0·90

The weighting scheme was $w = K/[f(F_o)]^2$, where $K = 0{\cdot}5618$ and $f(F_o) = 2{\cdot}63 - 0{\cdot}417|F_o|$ when $|F_o| < 2{\cdot}0$, $f(F_o) = 1{\cdot}69 + 0{\cdot}088$ when $2{\cdot}0 \le |F_o| < 7{\cdot}1$, $f(F_o) = 3{\cdot}51 - 0{\cdot}163$ when $7{\cdot}1 \le |F_o| < 13{\cdot}1$, $f(F_o) = 1{\cdot}42 - 0{\cdot}004$ when $13{\cdot}1 \le |F_o| < 29{\cdot}85$ and $f(F_o) = 1{\cdot}60 + 0{\cdot}000$ when $29{\cdot}85 \le |F_o| < 59{\cdot}74$. This weighting scheme produced $\langle w(F_o - F_c)^2 \rangle$ values independent of $\sin\theta$ and of the magnitudes of F_o. In the last cycle of refinement the R' value was 0·069 [$R' = \sqrt{(\Sigma\, w\Delta^2/\Sigma\, wF_o^2)}$].

The final atomic parameters for non-hydrogen atoms are listed in Table 2.* Table 3 gives the atomic parameters and bond distances for H atoms. All the computations were performed on a Univac 1108 computer.

Results and description of the structure

The conformation of the molecule is illustrated in Fig. 1. Final bond distances and angles are given in Table 4. The bond lengths in ifosfamide are compared with those for endoxan (cyclophosphamide) and trofosfamide in Table 5. The phosphamide groups exhibit an identical structure. The P=O group is in an axial position in each case, whereas the position of the other P substituent, N(1), is equatorial However, the most striking feature of these results is that R_3 in ifosfamide, as well as in trofosfamide, is also in an equatorial position (see Table 6, the deviations of atoms not included in the planes I and II). This is in good agreement with the results reported by Bentrude, Han-Van Tan & Yee (1974), showing that the amino-

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32337 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

group substituents are preferably in equatorial rather than in axial positions.

Least-squares planes through selected fragments of the molecule were computed (Table 6). The hetero ring has a chair conformation. The N atoms present a pyramidal non-planar conformation, N(1) being further from the plane than N(2) with deviations of −0·2436 and −0·2238 Å respectively.

The molecular packing and the hydrogen-bonding scheme are shown in Fig. 2. The only hydrogen bond present is the one between N(1) and O(1), 2·95 Å. Every molecule is linked by hydrogen bonds to a molecule related by a glide plane. Infinite chains or ribbons are formed in this way.

The conformation of the chloroethylamine groups is given through the torsion angles (Table 7). Both β-chloroethyl chains are extended and they move apart

Table 4. *Bond distances* (Å) *and angles* (°)

Cl(1)–C(1)	1·794 (9)	O(1)–P–O(2)	114·0 (3)
Cl(2)–C(2)	1·843 (9)	O(1)–P–N(1)	110·2 (3)
P–O(1)	1·475 (6)	O(2)–P–N(2)	102·2 (3)
P–O(2)	1·584 (5)	N(1)–P–N(2)	106·2 (3)
P–N(1)	1·631 (6)	O(1)–P–N(2)	117·6 (3)
P–N(2)	1·651 (6)	O(2)–P–N(1)	105·7 (3)
N(1)–C(2)	1·462 (9)	P–N(1)–C(2)	121·8 (5)
N(2)–C(3)	1·485 (9)	P–N(2)–C(3)	119·3 (5)
N(2)–C(5)	1·476 (9)	P–N(2)–C(5)	119·7 (5)
C(1)–C(2)	1·483 (10)	C(3)–N(2)–C(5)	114·5 (6)
C(3)–C(4)	1·472 (12)	Cl(1)–C(1)–C(2)	110·4 (6)
C(5)–C(6)	1·519 (11)	C(1)–C(2)–N(1)	108·9 (7)
C(6)–C(7)	1·512 (11)	N(2)–C(3)–C(4)	109·4 (7)
C(7)–O(2)	1·452 (9)	Cl(2)–C(4)–C(3)	107·8 (7)
		N(2)–C(5)–C(6)	111·9 (6)
		C(5)–C(6)–C(7)	111·2 (6)
		C(6)–C(7)–O(2)	108·6 (6)
		C(7)–O(2)–P	120·9 (5)
		P–N(1)–H(15)	109·9 (5)
		C(2)–N(1)–H(15)	118·2 (6)

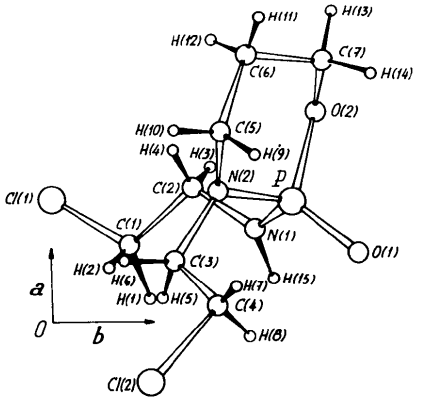


Fig. 1. The ifosfamide molecule.

Table 6. *Least-squares planes and deviations* (Å) *of the atoms*

The equations of the planes are expressed in orthogonal space as $PI + QJ + RK = S$

Plane I $-0{\cdot}5465I + 0{\cdot}7212J + 0{\cdot}4256K = 2{\cdot}2999$
Plane II $-0{\cdot}0989I + 0{\cdot}8287J + 0{\cdot}5509K = 4{\cdot}5894$
Plane III $-0{\cdot}2849I + 0{\cdot}4536J - 0{\cdot}8444K = 0{\cdot}3483$
Plane IV $-0{\cdot}5531I + 0{\cdot}3328J - 0{\cdot}7638K = -2{\cdot}7762$
Plane V $0{\cdot}7560I + 0{\cdot}5410J - 0{\cdot}3648K = 2{\cdot}9997$
Plane VI $-0{\cdot}4462I + 0{\cdot}6427J + 0{\cdot}6228K = 3{\cdot}2263$

Plane I		Plane II	
C(7)	0·0369	O(2)	0·0273
C(5)	−0·0362	P	−0·0242
O(2)	−0·0368	C(6)	−0·0281
N(2)	0·0360	C(5)	0·0250
P*	0·5943	N(2)*	−0·5079
C(6)*	−0·6653	C(7)*	0·6753
O(1)*	2·0486	C(3)*	−0·8614
N(1)*	−0·1819		
Plane III		**Plane IV**	
Cl(1)	0·0460	Cl(2)	−0·0405
C(1)	−0·0472	C(4)	0·0383
C(2)	−0·0548	C(3)	0·0535
N(1)	0·0560	N(2)	−0·0513
Plane V		**Plane VI**	
P	0·0000	P	0·0000
C(2)	0·0000	C(3)	0·0000
H(15)	0·0000	C(5)	0·0000
N(1)*	−0·2436	N(2)*	−0·2338

* Not included in the calculation of the plane.

Table 5. *Comparison with related compounds*

	Endoxan	Ifosfamide	Trofosfamide
	$C_7H_{15}N_2O_2PCl_2.H_2O$	$C_7H_{15}N_2O_2PCl_2$	$C_9H_{18}N_2O_2PCl_3$
P=O	1·470 (4) Å	1·475 (6) Å	1·468 (8) Å
P–O	1·582 (4)	1·584 (5)	1·585 (4)
P–N(1)	1·630 (4)	1·631 (6)	1·627 (9)
P–N(2)	1·625 (5)	1·651 (6)	1·656 (5)
C–Cl	1·784 (6)–1·794 (6)	1·794 (9)–1·843 (9)	1·780 (9)–1·793 (8)–1·846 (8)

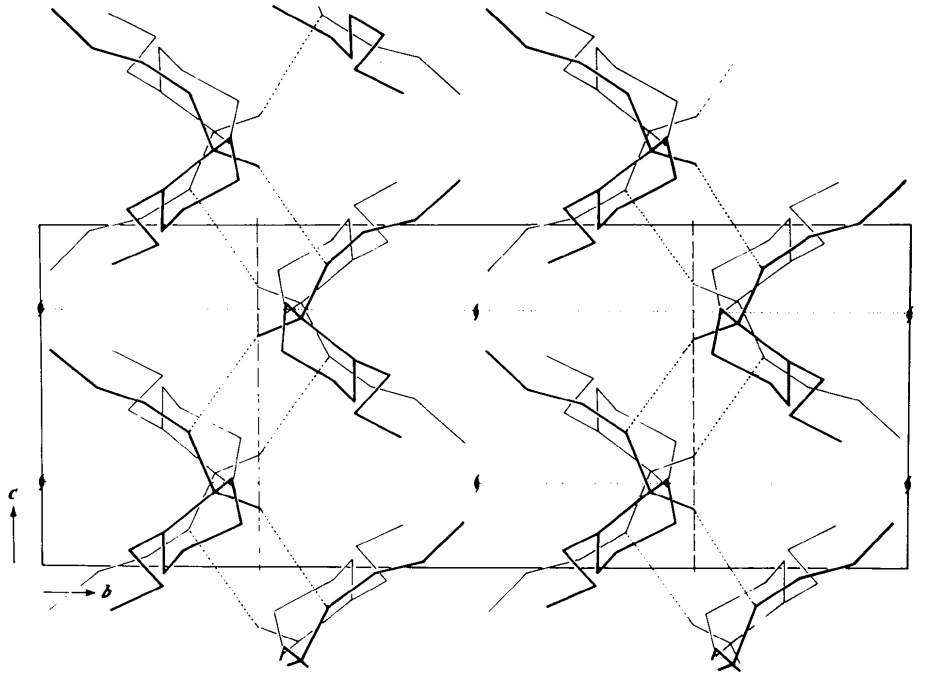


Fig. 2. Molecular packing and hydrogen bonding as viewed down **a**.

from each other. The distance Cl(1)—Cl(2) is 7·32 Å. On the other hand, the Cl—Cl distances in the three related compounds are: cyclophosphamide (endoxan) Cl(1)—Cl(2) = 9·01 Å; ifosfamide Cl(1)—Cl(2) = 7·32 Å; trofosfamide Cl(1)—Cl(2) = 6·8, Cl(1)—Cl(3) = 8·4, Cl(2)—Cl(3) = 7·4 Å.

An attempt to relate the chemical reactions of the alkylating agents to their biological effects has been made on those systems which possess two alkylating groups, which accounts for their ability to cross-link fibrous macromolecules. Brookes & Lawley (1960, 1961) reported that the sole site of alkylation of nucleic acids by mustard gas is at N(7) of guanine moieties. This has been actually confirmed for a number of alkylating agents. The model proposed by Crick & Watson (1954) for the structure of DNA shows that N(7) atoms of guanine moieties are sterically available for reactions. Thus a pair of such N(7) atoms could be linked through a chain of four or five atoms, *i.e.* a chain 8 Å long. According to the above-mentioned model, separation of the twin strands is required for cell division. This would in fact be inhibited if the strands were joined through a covalent bond as proposed. The above results suggest that the three compounds are able to yield cross-links between the N(7) guanine moieties, thus indicating the potential power of this research line.

Table 7. *Conformation about the β-chloroethylamine chains*

O(1)–P–N(1)–C(2)	−175·8 (5)°
P–N(1)–C(2)–C(1)	−128·4 (6)
N(1)–C(2)–C(1)–Cl(1)	−171·9 (4)
O(1)–P–N(2)–C(3)	−63·6 (6)
P–N(2)–C(3)–C(4)	76·6 (7)
N(2)–C(3)–C(4)–Cl(2)	173·1 (4)

We wish to thank Asta-Werke Laboratories for providing the material for this study, and acknowledge the assistance of Centro de Procesos de Datos del Ministerio de Educación y Ciencia.

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The Crystal and Molecular Structure of Trofosfamide {3-(2-Chloroethyl)-2-[bis (2-chloroethyl)amino]perhydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide, $C_9H_{18}Cl_3N_2O_2P$}

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Crystals of trosfosfamide are monoclinic, space group $P2_1/c$ with cell dimensions: $a = 13{\cdot}701$, $b = 13{\cdot}913$, $c = 8{\cdot}019$ Å and $\beta = 100{\cdot}36°$, with $Z = 4$. The crystal and molecular structure has been determined by single-crystal X-ray diffraction analysis. The structure was solved by direct methods and Fourier difference synthesis. The parameters were refined by full-matrix least-squares methods with individual anisotropic temperature factors to give the final R value of 0·074 for 2077 observed reflexions. Significant short distances of the P—N bonds are observed. Van der Waals interactions provide for the packing of the crystals.

Introduction

Trofosfamide, or ixoten, (Fig. 1) was developed in the research Laboratories of Asta-Werke, Wetsfallen (W. Germany). Trofosfamide is chemically a congener of endoxan (cyclophosphamide); its main advantage is its wide safety margin and consequently its markedly good tolerance to oral administration. Chemosensitive tumours, such as haemoblastoses and lymphoreticular tumours, may be treated with trofosfamide as initial therapy for induction of tumour remission and subsequently treated with the same drug to maintain remission.

Similarly to endoxan, trofosfamide is a primarily inactive transport form, thereafter to become activated in the body of the patient. The present study was undertaken to obtain a deeper insight into the configuration of this relevant drug.

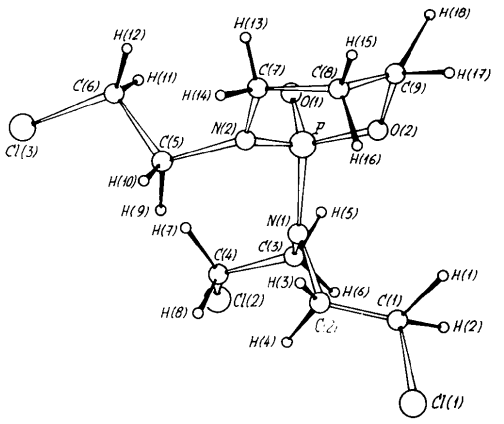

Fig. 1. The trofosfamide molecule.

Experimental

A powder sample of trofosfamide was kindly provided by Asta-Werke Laboratories. The sample was crystallized from an ether solution prepared by means of ether extraction in a Soxhlet apparatus, and subsequent cooling of the solution to a temperature of −10°C. The crystal data are collected in Table 1. Preliminary Weissenberg and precession photographs obtained with Cu $K\alpha$ radiation showed that the crystals are monoclinic, symmetry $2/m$, and systematic absences $0k0$ with k odd and $h0l$ with l odd suggested the space group $P2_1/c$. The intensity data were collected on an automatic four-circle Philips PW 1100 diffractometer with graphite-monochromatized Mo $K\alpha$ radiation and the $\omega/2\theta$ scan mode to a limit of $2\theta = 25°$; 2656 independent reflexions were measured of which 2077 had intensities greater than 2σ, where $\sigma^2(I) = C_p + C_b + (0{\cdot}04)^2 I^2$. C_p and C_b are peak and background counts respectively. Reflexions with intensities less than 2σ