X-Ray Crystallographic Determination of the Molecular Structures of *cis*- and *trans*-Isomers of (±)-5-Fluorocyclophosphamide {2-[Bis-(2-chloroethyl)amino]-5-fluorotetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide]}

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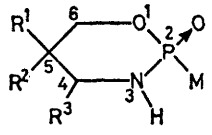
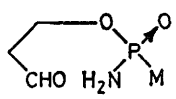
The crystal and molecular structures of the *cis*- and *trans*-isomers of (±)-5-fluorocyclophosphamide have been determined by *X*-ray methods. The two independent molecules of the *trans*-isomer have almost identical conformations, with a distorted half-chair ring pucker. The *cis*-isomer has a boat ring conformation.

CYCLOPHOSPHAMIDE {2-[bis-(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide} (1) is used widely as an alkylating-type anti-cancer drug. The drug requires metabolic activation [1] to the 4-hydroxy-derivative (2) which rapidly equilibrates with the acyclic tautomer, aldophosphamide (3), and undergoes β-elimination of acrolein to yield the reactive, alkylating species, phosphoramide mustard (PAM) (4). The importance of the formation of PAM to the anti-tumour activity of cyclophosphamide was demonstrated by 5,5-dideuteriation (5) which significantly reduced the rate of β-elimination [isotope effect (k_H/k_D) *ca.* 5] and also the potency and therapeutic index. In seeking to accelerate the rate of the β-elimination leading to PAM (4) the effect of introducing a highly electron-withdrawing substituent, namely fluorine, at position 5 was investigated. The synthesis, metabolism, and anti-tumour properties of *cis*- and *trans*-5-fluorocyclophosphamide (FCPA) (6) and (7), respectively, will be described elsewhere.[2] The susceptibility of (6) and (7) to metabolic attack differed markedly (4 and 46% respective disappearance after 50 min microsomal incubation) and, in view of the failure of n.m.r. [^{1}H (400 MHz), ^{13}C, and ^{19}F] studies to unequivocally establish the identity of the two geometrical isomers, single crystal *X*-ray analyses were carried out.



M=N(CH$_2$CH$_2$Cl)$_2$

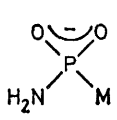


(3)

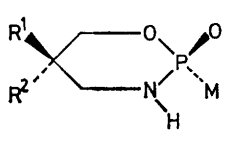
(1) R^1=R^2=R^3=H

(2) R^1=R^2=H, R^3=OH

(5) R^1=R^2=D, R^3=H



(4)



(6) R^1=F, R^2=H *cis*

(7) R^1=H, R^2=F *trans*

EXPERIMENTAL

trans-(±)-*FCPA*.—Crystals, obtained from diethyl ether, were colourless plates. *X*-Ray photography showed that they had monoclinic symmetry, and were uniquely assigned the space group $P2_1/n$. Accurate cell dimensions were obtained from least-squares refinement of 25 θ values measured on an Enraf–Nonius CAD4 diffractometer. These were a = 10.004(5), b = 10.751(1), c = 22.152(5) Å, β = 92.44(3)°. The observed density of 1.67 g cm^{-3} (by flotation), corresponds to eight molecules per unit cell, and therefore to two independent molecules per asymmetric unit.

The intensities of 4 058 unique reflections were measured on a diffractometer with graphite-monochromated Cu-K_α radiation ($1.5 < \theta < 65.0°$) using an ω–2θ scan technique. A periodic check on two selected reflections showed no significant crystal decomposition to have taken place during data collection. A total of 2 130 reflections were considered to have significant intensities [$F_o < 4\sigma(F_o)$] and were used in the subsequent analysis.

The structure was solved by direct methods, and refined by full-matrix least-squares techniques to a final reliability index R of 0.072, and a weighted R_w of 0.076. The positions of all hydrogen atoms (except for those attached to nitrogen atoms) were calculated from geometric considerations; these latter ones were located in a difference Fourier synthesis. All hydrogen atom parameters were kept fixed during the refinement. Table 1 details the final non-hydrogen positional parameters. Tables of thermal parameters, hydrogen atom co-ordinates, and observed and calculated structure factors, are available as Supplementary Publication No. SUP 23059/22 pp.).*

* For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc., Perkin Trans. 2*, 1980, Index issue.

cis-(±)-*FCPA*.—Crystals, also from diethyl ether solution, were flat plates that rapidly decomposed in air, and were accordingly sealed in capillary tubes. Diffraction photographs showed them to have orthorhombic symmetry; the space group assignment of *Pbcn* was confirmed by the subsequent structure analysis. Cell dimensions were

TABLE 1

Final fractional unit-cell co-ordinates for the non-hydrogen atoms of *trans*-FCPA. Estimated standard deviations are in parentheses

(a) Molecule 1

Atom	*x*	*y*	*z*
P(2)	0.374 8(2)	0.186 4(2)	0.181 6(1)
N(4)	0.393 0(7)	0.336 5(6)	0.183 0(3)
N(3)	0.503 5(7)	0.114 6(6)	0.155 5(3)
O(2)	0.337 2(5)	0.141 2(5)	0.240 9(2)
O(1)	0.265 5(5)	0.170 5(5)	0.127 7(2)
C(4)	0.510 4(10)	0.045 5(9)	0.100 3(4)
C(5)	0.384 9(10)	0.039 6(8)	0.061 3(4)
C(6)	0.261 8(9)	0.053 1(8)	0.095 2(4)
C(7′)	0.408 4(8)	0.404 7(7)	0.241 2(3)
C(8′)	0.553 5(9)	0.409 8(7)	0.262 1(3)
C(7)	0.430 4(9)	0.406 9(7)	0.129 2(3)
C(8)	0.310 4(9)	0.479 9(8)	0.105 3(4)
Cl(1′)	0.567 1(3)	0.485 8(2)	0.334 2(1)
Cl(1)	0.364 6(3)	0.578 6(2)	0.044 5(1)
F(1)	0.388 2(8)	0.131 4(7)	0.017 9(2)

(b) Molecule 2

P(2)	0.132 8(2)	0.453 2(2)	−0.173 9(1)
N(4)	0.110 8(7)	0.603 7(6)	−0.174 6(3)
N(3)	0.006 1(7)	0.377 8(6)	−0.149 2(3)
O(2)	0.171 7(5)	0.412 0(5)	−0.233 5(2)
O(1)	0.239 5(5)	0.437 4(5)	−0.120 4(2)
C(4)	−0.007 0(9)	0.318 0(9)	−0.091 7(4)
C(5)	0.117 1(9)	0.303 7(9)	−0.053 9(4)
C(6)	0.243 8(9)	0.319 3(8)	−0.087 3(4)
C(7′)	0.096 8(8)	0.672 8(7)	−0.233 0(4)
C(8′)	−0.049 6(9)	0.678 2(8)	−0.253 4(3)
C(7)	0.072 7(9)	0.670 1(8)	−0.119 8(3)
C(8)	0.191 2(9)	0.738 9(8)	−0.093 9(4)
Cl(1′)	−0.061 6(3)	0.751 8(2)	−0.325 5(1)
Cl(1)	0.141 4(3)	0.843 4(2)	−0.034 9(1)
F(1)	0.116 3(8)	0.390 9(10)	−0.009 6(3)

obtained by least-squares analysis of 25 θ values measured diffractometrically, and are $a = 23.162(2)$, $b = 12.971(4)$, $c = 9.203(1)$ Å. A crystal density was not able to be measured, but assigning eight molecules to the unit cell (*i.e.* one per asymmetric unit) gives a calculated density of 1.69 g cm^{-3}.

The intensities of 2 278 reflections were estimated on the diffractometer with graphite-monochromated Cu-K_α radiation ($1.5 < \theta < 65.0$) with the ω–2θ scan technique. Three standard reflections were monitored for crystal decay at an interval of every 100 reflections. These showed a maximal 60% crystal decay at the end of data collection, and the observed intensities were corrected accordingly for this severe decay. Due to the lack of a supply of other suitable crystals, it was not possible to substitute the procedure used for one in which data from a number of crystals would be measured. A total of 1 258 reflections were judged to have intensity significant at the 4σ level, and were used in the subsequent analysis.

Routine application of direct methods procedures enabled the positional co-ordinates of all non-hydrogen atoms of the fluorocyclophosphamide moiety to be defined. Subsequent Fourier and difference Fourier syntheses revealed a region of diffuse electron density around the origin of the unit cell. This was presumed to correspond to the presence of solvated ether; however it did not prove possible to assign the peaks in this region unequivocally to either a single sensible atomic geometry, or to a set of disorder models for the solvent. Further attempts at this were therefore abandoned, and refinement was subsequently performed on the FCPA molecule alone. Such a procedure was considered justified in view of the attainment of the objective of the study, *viz.* an assignment of the geometry of the FCPA molecule.

The structure was refined by full-matrix least-squares methods to a final R of 0.123 and a weighted R_w of 0.130. Hydrogen atom positions were calculated from geometric considerations, and were kept fixed during the refinement. Table 2 details the non-hydrogen positional parameters. Other Tables, as for *trans*-(±)-FCPA, are available in SUP 23059.

TABLE 2

Final fractional unit-cell co-ordinates for *cis*-FCPA

Atom	*x*	*y*	*z*
P(2)	0.220 6(2)	0.358 6(3)	0.773 3(3)
N(4)	0.157 7(4)	0.337 1(8)	0.706 9(11)
N(3)	0.267 6(5)	0.346 1(10)	0.639 9(11)
O(2)	0.228 6(4)	0.295 7(7)	0.907 4(9)
O(1)	0.227 8(4)	0.476 5(7)	0.815 3(8)
C(4)	0.315 7(7)	0.404 1(16)	0.625 5(17)
C(5)	0.307 6(7)	0.521 3(14)	0.664 4(17)
C(6)	0.250 0(8)	0.549 4(10)	0.712 6(16)
C(7′)	0.109 3(7)	0.297 6(14)	0.795 3(15)
C(8′)	0.080 5(8)	0.378 8(18)	0.875 3(20)
C(7)	0.139 5(7)	0.376 3(12)	0.560 2(14)
C(8)	0.133 9(9)	0.274 9(14)	0.456 1(15)
Cl(1′)	0.021 1(2)	0.335 3(7)	0.974 2(5)
Cl(1)	0.105 6(2)	0.325 5(4)	0.286 4(4)
F(1)	0.346 2(5)	0.545 8(13)	0.765 8(16)

Calculations were performed on the University of London CDC7600 computer, and on a PDP 11/34 system using the Enraf–Nonius structure determination package.

RESULTS AND DISCUSSION

Figures 1—3 show various views of the molecular structures determined in this study. Tables 3 and 4 detail relevant bond lengths and angles. Both isomers

TABLE 3

Bond lengths (Å). Estimated standard deviations are in parentheses

	trans-FCPA		
	Molecule 1	Molecule 2	*cis*-FCPA
O(1)–P(2)	1.593(5)	1.571(5)	1.58(1)
O(1)–C(6)	1.452(10)	1.466(10)	1.43(2)
P(2)–O(2)	1.465(5)	1.461(5)	1.49(1)
P(2)–N(3)	1.628(7)	1.620(7)	1.65(1)
P(2)–N(4)	1.624(6)	1.633(6)	1.60(1)
N(3)–C(4)	1.435(11)	1.438(10)	1.35(2)
C(4)–C(5)	1.495(13)	1.476(12)	1.57(3)
C(5)–F(1)	1.379(11)	1.358(12)	1.33(2)
C(5)–C(6)	1.476(14)	1.503(13)	1.45(3)
N(4)–C(7)	1.475(10)	1.493(10)	1.50(2)
N(4)–C(7′)	1.485(9)	1.472(10)	1.48(2)
C(7)–C(8)	1.511(12)	1.516(12)	1.63(2)
C(7′)–C(8′)	1.505(12)	1.491(12)	1.45(3)
C(8)–Cl(1)	1.815(9)	1.811(9)	1.82(2)
C(8′)–Cl(1′)	1.794(8)	1.782(8)	1.74(2)

exist in the crystal as racemates; therefore, absolute configurations have not been determined in this study.

The existence of two crystallographically independent molecules of *trans*-FCPA in effect means that the molecular structure of this isomer has been determined twice. Since *a priori* there is no reason for these two molecules to be related in structural terms, the observations (elaborated in more detail below) that they are indeed very similar strongly implies that the observed molecular geometries and conformations are intrinsic properties

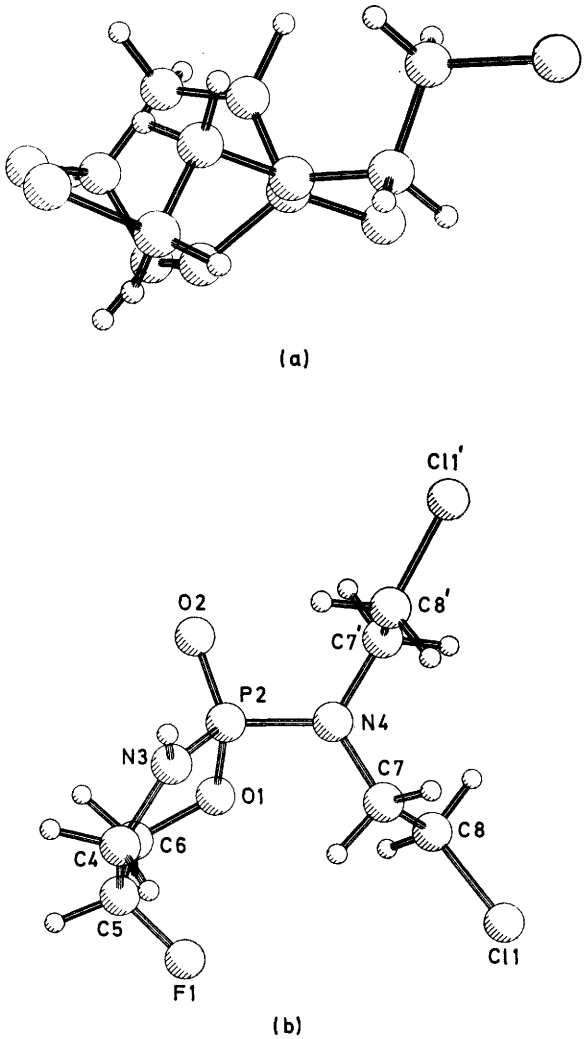


FIGURE 1 Two computer-drawn views of the molecular structure of *trans*-FCPA, molecule 1. (a), Projected approximately along the phosphorus–nitrogen bond; (b), rotated by 90° in vertical from (a). The smaller circles represent hydrogen atoms

of the molecule, and are not artifacts resulting for example, from crystal packing forces. The relatively poorly defined geometry of the *cis*-isomer structure is undoubtedly due to the crystal instability caused by continuous and uncontrollable loss of solvent. Nevertheless, the analysis of this isomer has enabled the important features of the structure to be unequivocally defined.

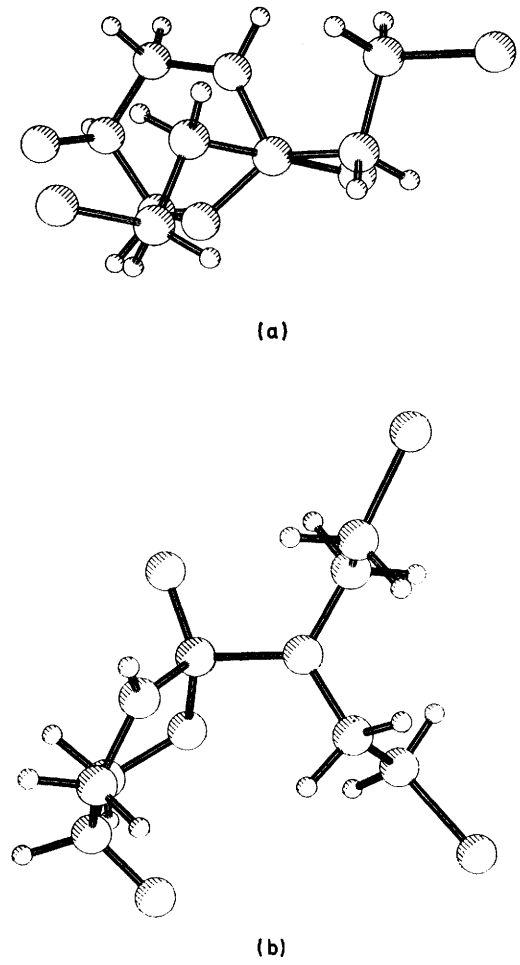


FIGURE 2 (a), (b), Two projections of molecule 2 of *trans*-FCPA, drawn from the same viewpoints as Figure 1

The two molecules of *trans*-FCPA (Figures 1 and 2) have very similar conformations, with in both cases a *trans*-disposition of the fluorine substituent on C(2),

TABLE 4

Bond angles (°). Estimated standard deviations are in parentheses

	trans-FCPA		
	Molecule 1	Molecule 2	*cis*-FCPA
P(2)–O(1)–C(6)	117.8(5)	118.4(5)	121(1)
O(1)–P(2)–N(3)	102.3(3)	101.9(3)	102(1)
O(1)–P(2)–O(2)	116.2(3)	116.8(3)	108(1)
O(1)–P(2)–N(4)	101.2(3)	101.6(3)	111(1)
O(2)–P(2)–N(3)	113.4(3)	113.4(3)	119(1)
O(2)–P(2)–N(4)	110.1(3)	109.5(3)	109(1)
N(3)–P(2)–N(4)	112.9(4)	113.1(4)	107(1)
P(2)–N(3)–C(4)	127.9(6)	128.7(6)	124(1)
N(3)–C(4)–C(5)	116.4(8)	116.3(7)	115(1)
C(4)–C(5)–C(6)	113.6(7)	114.6(7)	115(1)
C(4)–C(5)–F(1)	109.2(8)	107.8(8)	108(1)
F(1)–C(5)–C(6)	109.2(8)	108.1(8)	110(1)
C(5)–C(6)–O(1)	109.5(7)	109.6(7)	111(1)
P(2)–N(4)–C(7)	121.7(5)	120.6(5)	123(1)
P(2)–N(4)–C(7′)	121.0(5)	120.8(5)	123(1)
C(7)–N(4)–C(7′)	115.4(6)	117.0(6)	114(1)
N(4)–C(7)–C(8)	109.0(7)	109.3(6)	106(1)
C(7)–C(8)–Cl(1)	107.8(6)	108.2(6)	104(1)
N(4)–C(7′)–C(8′)	110.4(6)	109.2(7)	112(1)
C(7′)–C(8′)–Cl(1′)	109.0(1)	110.3(6)	113(1)

relative to the mustard [*i.e.* bis(chloroethyl)amino] side-chain. The six-membered oxazaphosphorinane ring adopts in both cases a distorted half-chair conformation (Table 5), in striking contrast to the standard chair structures observed in cyclophosphamide itself.[3-6]

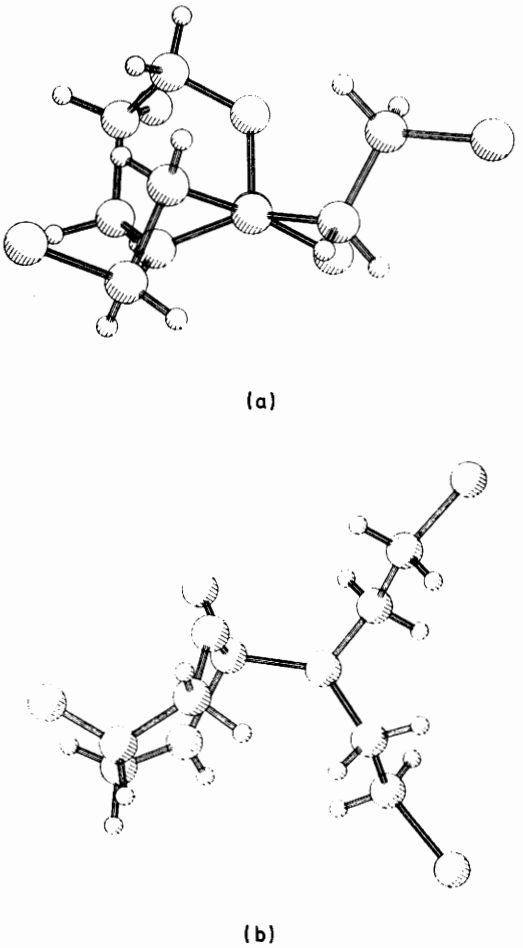


FIGURE 3 Two projections of the *cis*-FCPA molecule

Chair conformations have also been observed in *cis*-4-phenylcyclophosphamide [7] and 4-hydroperoxycyclophosphamide;[8] that in 4-oxocyclophosphamide[9] is unsurprisingly buckled. In both *cis*-FCPA molecules, the mustard side-chain is approximately axial, and the fluorine atom is equatorial to the ring. All cyclophosphamide and derivative structures,[3-6,8,9] apart from the *cis*-4-phenyl one,[7] have the former as an equatorial substituent. The *cis*-FCPA isomer in contrast (Figure 3) has a boat conformation for its six-membered ring, with N(2) and C(3) as 'prow' and 'stern'. In this structure, both the mustard side-chain and the fluorine atom are equatorial to the ring, resulting in them being *trans* to each other.

All three molecular arrangements determined in this study have their mustard side-chains fully extended, with approximate two-fold symmetry along the P(1)–N(1) axis. Such an arrangement is common in this series.[3-9] Bond lengths and angles in the two isomers are in general, unexceptional. Those in the two crystallographically independent molecules of *trans*-(±)-FCPA are equivalent within experimental error, and compare reasonably well with the majority of bond geometry values in the rather less well defined *cis*-isomer. In this latter structure, the principle deviants from this pattern are the bond lengths C(4)–C(5) (1.57 Å), N(3)–C(4) (1.35 Å), C(7)–C(8) (1.63 Å), and C(8')–C(11') (1.74 Å). These distances are the only ones that are significantly different from those previously reported for racemic cyclophosphamide,[3,4] and for its (−)-optical isomer.[6] The accuracy of the analysis [3] of (+)-cyclophosphamide [5] was hindered by crystal decomposition during data collection, and it is therefore unsurprising that several abnormal bond distances and angles are reported for this structure, paralleling our results with *cis*-FCPA.

The P(2)–O(2) bond distance of 1.472 Å, averaged over the two independent *trans*-FCPA molecules and the *cis*-one, compares well with that found in (±)-cyclophosphamide [1.470(4) [3] and 1.466(4) Å [4]], in the (+)-isomer [1.53(2) Å [5], and in the (−)-isomer [1.48(1) Å, averaged over three independent molecules.[6]] The average P(2)–O(1) distance here of 1.583 Å is similarly in accord with the corresponding bond length in these other structures. These phosphorus–oxygen bond lengths are thus in accord with an assignment of double- and single-bond character to P(2)–O(2) and P(2)–O(1) respectively, so that the introduction of the highly electronegative fluorine atom at C(5) has not altered the electron distribution around the phosphorus atom. This is confirmed by the normality of the P–N bonds (with an average length of 1.627 Å). The bond geometry in the immediate vicinity of the fluorine substituent in these compounds is similarly unperturbed

TABLE 5

Ring torsion angles (°) in the two crystallographically independent molecules of *trans*-FCPA, in *cis*-FCPA, and in the crystal structures of cyclophosphamide (CPA)

Angle	*trans*-FCPA Molecule 1	*trans*-FCPA Molecule 2	*cis*-FCPA	(+)-CPA [3]	(−)-CPA [4]	Average of results from refs. 1 and 2
O(1),P(2),N(3),C(4)	−5	3	27	38	−40	38
P(2),N(3),C(4),C(5)	1	−12	−39	−42	44	−47
N(3),C(4),C(5),C(6)	−27	−17	1	50	−53	55
C(4),C(5),C(6),O(1)	58	53	45	−60	62	−62
C(5),C(6),O(1),P(2)	−67	−66	−59	63	−64	60
C(6),O(1),P(2),N(3)	38	36	24	−50	50	−45

relative to cyclophosphamide itself, disregarding the clearly abnormally long C(4)–C(5) bond length in *cis*-FCPA. As in cyclophosphamide structures, bond distances C(4)–C(5) and C(5)–C(6) are equivalent within experimental error. There is however, some evidence of a slight increase in the C(4)–C(5)–C(6) bond angle by about 2°, as a consequence of fluorination at C(5). The average C(5)–F bond distance of 1.36 Å is well within the accepted range of values for this type of bond.[10]

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