The arsonomethyl group as an analogue of phosphate

An X-ray investigation

Kazuhide KAMIYA,* William B. T. CRUSE and Olga KENNARD

University of Cambridge Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

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The X-ray structure analysis of three compounds of interest as enzyme substrates is reported. They are the hydrated forms of (I) DL-2-amino-4-arsonobutanoic acid [$\text{HO}-\text{AsO}_2^--\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{CO}_2\text{H}$], (II) DL-2-amino-4-phosphonobutanoic acid [$\text{HO}-\text{PO}_2^--\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{CO}_2\text{H}$] and the hydrated barium salt of (III) D-3-phosphoglycerate [$\text{HO}-\text{PO}_2^--\text{O}-\text{CH}_2-\text{CH}(\text{OH})-\text{CO}_2^-$]. The structures were fully refined to *R* factors of 0.033, 0.053 and 0.046. For the compounds (I) and (II) the charge distribution was directly determined by locating all H atoms. The co-ordination around As and P is approximately tetrahedral, with the valency angle between the two charged O atoms enlarged to 112° in compound (I), 166° in compound (II) and 122° in compound (III). The As–X bond distances are increased relative to P–X to accommodate the increased atomic radius. The analysis establishes that the compounds are structural analogues. Tables of co-ordinates for H atoms, anisotropic thermal parameters, bond lengths and bond angles for the three compounds have been deposited as Supplementary Publication SUP 50122 (5 pages) with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained directly [see *Biochem J.* (1983) 209, 5].

Compounds containing the $-\text{CH}_2-\text{PO}_3\text{H}_2$ group in place of the $-\text{O}-\text{PO}_3\text{H}_2$ group have long been used in biochemical studies of analogues of natural substrates. Substrate analogues containing the $-\text{O}-\text{AsO}_3\text{H}_2$ group have also been of interest, since arsenate is a good analogue of phosphate in enzymic reactions. The use of this group, however, is limited by the ease of the hydrolysis of esters of arsenic acid. An alternative is to consider the behaviour of phosphate analogues that contain the $-\text{CH}_2-\text{AsO}_3\text{H}_2$ group, especially because any enzymic reaction that phosphorylates this group would be followed by hydrolysis of the product (Webster *et al.*, 1978). For this reason Falvello *et al.* (1977) studied the X-ray structure of arsonolmethylphosphonic acid ($\text{H}_2\text{O}_3\text{As}-\text{CH}_2-\text{PO}_3\text{H}_2$) and showed the close similarity between the $-\text{CH}_2-\text{PO}_3\text{H}_2$ and $-\text{OPO}_3\text{H}_2$, which was reviewed by Yount (1975). The present study extends the comparison of these groups by the X-ray analysis of three compounds, $\text{HO}-\text{AsO}_2^--\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{CO}_2\text{H}$ monohydrate, $\text{HO}-\text{PO}_2^--\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{CO}_2\text{H}$ monohydrate and the barium salt of $\text{HO}-\text{PO}_2^--\text{O}-\text{CH}_2-\text{CH}(\text{OH})-\text{CO}_2^-$ dihydrate, which are of interest as enzyme substrates.

* On leave from Takeda Chemical Industries Ltd., Osaka, Japan.

Structure of DL-2-amino-4-arsonobutanoic acid monohydrate (I)

Crystal data

The crystal data for $\text{C}_4\text{H}_{10}\text{AsNO}_5\cdot\text{H}_2\text{O}$ ($M=245.06$) are as follows: monoclinic, $a=1.0330(2)$, $b=1.6581(3)$, $c=0.49662(7)$ nm; $\beta=107.31(1)^\circ$; $V=0.8121(2)$ nm³; $D_x=2.0$ Mg·m^{−3}; $Z=4$; space group $P2_1/a$ from absences; $\lambda=0.15418$ nm; $\mu(\text{Cu } K_\alpha)=5.4$ mm^{−1}.

Experimental

A colourless crystal, prepared as described in the accompanying paper (Adams *et al.*, 1983), of dimensions 0.2 mm × 0.25 mm × 0.45 mm was used for the data collection on a Syntex P2₁ diffractometer with graphite monochromatized Cu K_α -radiation. Cell dimensions were determined by least-squares fit on the setting angles of 25 reflexions. The intensities of 1379 unique reflexions with $2\theta < 130^\circ$ were measured in the $\omega/2\theta$ scan mode. Lorentz, polarization and empirical absorption corrections were applied. A total of 1365 structure factors with $F > 3\sigma(F_0)$ were used in the analysis.

Structure solution and refinement

The position of the As atom at 0.312, 0.099,

Table 1. Fractional atomic co-ordinates for DL-2-amino-4-arsonobutanoic acid monohydrate

	$10^4 \times x/a$	$10^4 \times y/b$	$10^4 \times z/c$
As-1	3122(0.5)	984(0.3)	2310(1)
O-3	4318(3)	854(2)	5554(6)
O-2	1793(3)	1478(2)	2828(6)
O-1	3793(3)	1476(2)	118(6)
O-4	-479(3)	-1040(2)	4011(8)
O-5	355(4)	-2279(2)	4424(10)
O-6	1423(3)	3075(2)	2080(7)
N-1	2261(4)	-1947(2)	1992(8)
C-1	326(4)	-1592(3)	3624(9)
C-2	1242(4)	-1308(2)	1910(9)
C-3	1912(5)	-502(3)	2996(9)
C-4	2607(4)	-99(3)	1009(9)

0.230 was deduced from a Patterson map. The electron-density map phased with As gave the location of all non-hydrogen atoms, including the O atom of the water of hydration. The structure was refined first isotropically and then anisotropically to $R = 0.043$. The H atoms, located initially from difference maps, were introduced in geometrically calculated positions and included in further cycles of full-matrix least-squares refinement with a common isotropic temperature factor. The refinement converged at $R = 0.033$. Table 1 lists the final refined values of the fractional atomic co-ordinates for the non-hydrogen atoms. Tables of co-ordinates for H atoms, anisotropic thermal parameters, bond lengths and bond angles for the three compounds described

in the present paper have been deposited as Supplementary Publication SUP 50122 with the British Library Lending Division.

Results

Fig. 1(a) shows the conformation of the molecule observed in the crystal structure. The molecule exists in the zwitterion form, with a delocalized negative charge around As–O-2 and As–O-3 and a positive charge on the N-1 atom. The carboxy group is not ionized, and the C-1–O-5 and C-1–O-4 bond distances are 0.1288(5) and 0.1204(6) nm respectively.

In the extended crystal structure all possible hydrogen donors and acceptors, with the exception of the carboxy O atom O-5, participate in hydrogen-bond formation. The interaction between the two charged groups is mediated by the water molecule of crystallization, which donates one hydrogen bond to each of the charged O atoms (O-1 and O-2) of two symmetry related AsO_2 residues and accepts one hydrogen bond from each of two related N atoms. Full details are given in Table 2, and the packing of the molecules is illustrated in Fig. 2.

Structure of DL-2-amino-4-phosphonobutanoic acid monohydrate (II)

Crystal data

The crystal data for $\text{C}_4\text{H}_{10}\text{NO}_5 \cdot \text{H}_2\text{O}$ ($M = 201.1$) are as follows: monoclinic, $a = 0.8416(1)$, $b = 0.4953(1)$, $c = 2.0602(3)$ nm; $\beta = 100.43(1)^\circ$; $V =$

Fig. 1. Comparison of the molecular conformation found in the crystal structures of (a) DL-2-amino-4-arsonobutanoic acid monohydrate (I), (b) DL-2-amino-4-phosphonobutanoic acid monohydrate (II) and (c) barium D-3-phosphoglycerate dihydrate (III)

The atom numbering schemes correspond to the co-ordinates in Tables 1, 2 and 5. To facilitate comparison the D-form is drawn for compounds (I) and (II).

Table 2. *Intermolecular hydrogen-bonding of DL-2-amino-4-arsonobutanoic acid monohydrate (I)*

H donor	H	H acceptor	Symmetry*	Distance donor-acceptor (nm)	Distance H-acceptor (nm)
O-1	H-1-O-1	O-3	($x, y, 1+z$)	0.269	0.174
O-4	H-1-O-4	O-2	($\bar{x}, \bar{y}, 1-z$)	0.247	0.159
O-6	H-1-O-6	O-3	($x-\frac{1}{2}, \frac{1}{2}-y, z$)	0.270	0.170
O-6	H-2-O-6	O-2	(x, y, z)	0.269	0.171
N-1	H-1-N-1	O-6	($\frac{1}{2}-x, y-\frac{1}{2}, \bar{z}$)	0.275	0.183
N-1	H-2-N-1	O-3	($\frac{1}{2}-x, \frac{1}{2}-y, \bar{z}$)	0.291	0.191
N-1	H-3-N-1	O-6	($\frac{1}{2}-x, y-\frac{1}{2}, 1-z$)	0.285	0.181

* Atoms of hydrogen acceptor belong to the molecule related by symmetries shown in this column.

Fig. 2. *Projection of the crystal structure of DL-2-amino-4-arsonobutanoic acid (I) on the a-b plane, showing the hydrogen-bonding scheme, the packing of the molecules in the crystal lattice and the hydrogen-bonding*

0.8446(4) nm³; $D_x = 1.58 \text{ Mg} \cdot \text{m}^{-3}$; $Z = 4$; space group $P2_1/c$; $\lambda = 0.15418 \text{ nm}$; $\mu (\text{Cu } K_\alpha) = 2.8 \text{ mm}^{-1}$.

Experimental

The experimental procedure was as described for compound (I) but with a crystal, as prepared by Dixon & Sparkes (1974), of dimensions 0.02 mm $\times$ 0.1 mm $\times$ 0.3 mm on a Siemens-Stoe 4 circle diffractometer and without the application of absorption corrections. Of the 1060 reflexions measured 829 had $F > 3\sigma (F_0)$ and were treated as observed.

Structure solution and refinement

The structure was solved by direct methods, and the positions of all non-hydrogen atoms except for the water O atom were derived from the first E map. The structure was refined anisotropically to $R = 0.074$, after which all H atoms were clearly indicated on the difference map. During the remainder of the refinement the H atoms attached to C atoms were included at calculated positions, and those attached to N and O atoms at the observed positions, but constrained in the refinement so that O-H and N-H distances were $0.10 \pm 0.01 \text{ nm}$. An overall isotropic temperature factor was used for H

Table 3. Fractional atomic co-ordinates for DL-2-amino-4-phosphonobutanoic acid monohydrate

	$10^4 \times x/a$	$10^4 \times y/b$	$10^4 \times z/c$
P-1	3099(2)	3218(3)	831(1)
O-1	2409(5)	623(9)	516(2)
O-2	2279(5)	4325(10)	1359(2)
O-3	3037(6)	5386(9)	260(2)
O-4	7049(6)	3013(11)	2792(2)
O-5	8890(5)	800(9)	2342(2)
O-6	792(6)	716(10)	9259(2)
N-1	8931(6)	4348(12)	1388(3)
C-1	7958(8)	2664(13)	2350(3)
C-2	7787(7)	4896(14)	1848(3)
C-3	6023(7)	5279(13)	1464(3)
C-4	5212(7)	2685(13)	1166(3)

atoms; refinement converged at $R = 0.053$. The final fractional atomic co-ordinates are given in Table 3.

Results

The structure (Fig. 1b) is very similar to that of compound (I). The molecule is in the zwitterion form, with a positively charged N atom and a delocalized negative charge distributed between two unprotonated O atoms of the HO-PO_2^- group. This interpretation is supported by the P–O bond-length values (Table 8). The carboxy group is not ionized, having C–1–O–4 and C–1–O–5 distances that are significantly different [0.1302(9) and 0.1213(9) nm]. The role of the water molecule in the extended crystal structure is similar to that of compound (I), except that the O atoms O-2 of two symmetry-related molecules function as the hydrogen-bond acceptors. Details are given in Table 4; the packing of the molecules in the unit cell is illustrated in Fig. 3.

Structure of barium D-3-phosphoglycerate dihydrate (III)

Crystal data

The crystal data for $\text{C}_3\text{H}_5\text{BaO}_7\text{P}_2\text{H}_2\text{O}$ ($M = 357.4$) are as follows: monoclinic, $a = 0.5391(2)$,

$b = 0.8213(3)$, $c = 1.0782(3)$ nm; $\beta = 100.52(2)^\circ$; $V = 0.4694$ nm³; $D_x = 2.53$ Mg · m⁻³; $Z = 2$; space group = P2_1 from absences; $\lambda = 0.15418$ nm, μ (Cu K_α) = 34 mm⁻¹.

Experimental

Barium D-3-phosphoglycerate (Koch–Light Laboratories) was converted into its bis(cyclohexylammonium) salt by stirring it in water with the H^+ form of a sulphonated polystyrene resin (Zerolit 225) and filtering through a bed of the same resin to obtain a solution of the free acid, which was adjusted to pH 5 with cyclohexylamine and evaporated to dryness. It was crystallized from methanol on addition of diethyl ether. Colourless plates of barium salt were obtained by slow diffusion of aqueous BaCl_2 into an aqueous solution of this bis(cyclohexylammonium) salt. Experimental conditions were as for compound (II) with a crystal of dimensions 0.1 mm $\times$ 0.2 mm $\times$ 0.01 mm. The intensities of 652 unique reflexions were measured, of which 642 had $F > 3\sigma$ (F_0) and were treated as observed. Absorption corrections, derived by using the observed shape of the crystal, were applied in addition to the Lorentz and polarization corrections.

Structure solution and refinement

The location of the Ba^{2+} ion was determined from a Patterson map, and the remaining non-hydrogen atoms, including those of two water molecules, were determined from difference maps. The H atoms bonded to C atoms were included in calculated positions with a fixed thermal parameter $U = 0.0004$ nm². In the final cycle of full-matrix least-squares refinement, atomic positions and thermal parameters (Ba, P-anisotropic; C, O-isotropic) were refined with a reflexion weighting scheme $w = [\sigma^2(F_0) + 0.0006F_0^2]^{-1}$. Owing to the dominant scattering of Ba and subsequent extinction effects, the remaining five H atoms could not be located from difference maps and anisotropic refinement of C and O atoms was not satisfactory. Refinement of the parameters for both enantiomers by using the

Table 4. Intermolecular hydrogen-bonding of DL-2-amino-4-phosphonobutanoic acid monohydrate (II)

H donor	H	H acceptor	Symmetry*	Distance donor–acceptor (nm)	Distance H–acceptor (nm)
O-3	H-1–O-3	O-1	($x, 1+y, z$)	0.227	0.173
O-4	H-1–O-4	O-2	($1-x, \frac{1}{2}-y, \frac{1}{2}-z$)	0.252	0.154
O-6	H-1–O-6	O-1	($x-1, y, z$)	0.270	0.173
O-6	H-2–O-6	O-1	($1-x, -y, -z$)	0.289	0.191
N-1	H-1–N-1	O-6	($1-x, 1-y, 1-z$)	0.281	0.187
N-1	H-2–N-1	O-6	($1-x, -y, 1-z$)	0.287	0.195
N-1	H-3–N-1	O-2	($1+x, y, z$)	0.283	0.205
N-1	H-3–N-1	O-5	($1+x, y, z$)	0.300	0.226

* Atoms of the hydrogen acceptor belong to the molecule related by symmetries shown in this column.

Fig. 3. Projection of the crystal structure of DL-2-amino-4-phosphonobutanoic acid (II) monohydrate about the *b*-axis, illustrating the packing of the molecules in the crystal lattice and the hydrogen-bonding

Table 5. Fractional atom co-ordinates and isotropic temperature factors for barium D-3-phosphoglycerate

	$10^4 \times x/a$	$10^4 \times y/b$	$10^4 \times z/c$	$10^6 \times U(\text{nm}^2)$
Ba-1	11221(1)	10000	4269(1)	
P-1	6083(10)	2454(8)	2749(4)	
O-1	8177(24)	2925(16)	3811(12)	22(3)
O-2	3412(23)	2330(21)	2953(11)	28(3)
O-3	5977(20)	3617(16)	1583(10)	28(3)
O-4	7011(20)	738(17)	2327(10)	22(2)
O-5	7922(25)	-2896(19)	3696(14)	28(3)
O-6	3802(21)	-2432(19)	3170(12)	23(3)
O-7	8811(25)	-2082(18)	1394(13)	41(4)
C-3	5470(23)	-304(27)	1330(11)	19(3)
C-2	6315(28)	-1965(23)	1597(14)	22(4)
C-1	6068(47)	-2507(35)	2937(21)	31(5)
O-8	3294(16)	4963(24)	4676(7)	24(2)
O-9	10280(25)	4171(20)	988(13)	49(3)

anomalous scattering for Ba gave the final residuals $R_R = 0.046$ and $R_S = 0.057$, which confirmed the anionic structure as (2*R*)-D-3-phosphoglycerate. Final fractional atomic co-ordinates are given in Table 5.

Results

The conformation of the D-3-phosphoglycerate anion is shown in Fig. 1(c). The individual values of the bond lengths and bond angles are less precise than in the previous two structures, because of the dominant scatter of the Ba^{2+} ion. The C–C and the

C–O distances are particularly affected and have standard deviations of 0.002–0.003 nm. The phosphate group is more precisely determined, with P–O bond lengths of 0.1505(14), 0.1499(13), 0.1571(13) and 0.1590(15) nm for atoms O-1 to O-4 respectively. The O-1–P–O-2 angle is 120.9(7)°; other O–P–O angles range from 111.4° to 102.4°. This geometry is consistent with the phosphate group carrying a single negative charge delocalized between O-1 and O-2. The second negative charge required for electrical neutrality cannot be assigned with confidence, although it would be expected to reside on the carboxyl substituent.

The positions of the H atoms bonded to O atoms could not be determined directly from difference maps. They are probably disordered, forming a variety of linear and bifurcated hydrogen bonds, as indicated by the large number of close O...O contacts between potential donors and acceptors (Table 6).

The extended crystal structure is principally stabilized by ionic interactions between charged species and by hydrogen-bonding between the molecules and the two molecules of water of crystallization. Only one hydrogen bond is involved in direct intermolecular contact between neighbouring molecules. The hydrogen-bonding pattern with donor–acceptor assignments is listed in Table 6, and the crystal structure is illustrated in Fig. 4. The environment of the Ba^{2+} ion, which is surrounded by nine O atoms, is described in Table 7.

Table 6. *Selected non-bonded distances for barium D-3-phosphoglycerate dihydrate*

Key to symmetry operations relating designated atoms to reference atoms at (x, y, z) : $^a 2.0-x, -0.5+y, 1.0-z$; $^b 1.0-x, 0.5+y, 1.0-z$; $^c 1.0+x, y, z$; $^d x, 1.0+y, z$; $^e 2.0-x, 0.5+y, -z$.

	Bond distance (nm)		Bond distance (nm)
O-5-O-1 ^a	0.3169	O-8-O-1 ^b	0.3111
O-8-O-2	0.2859	O-9-O-2 ^c	0.2885
O-9-O-3	0.2558	O-7-O-5	0.2696
O-8-O-5 ^b	0.3074	O-7-O-6 ^c	0.3018
O-8-O-6 ^d	0.2729	O-9-O-7 ^e	0.2890

Table 7. *Ba²⁺ co-ordination in barium D-3-phosphoglycerate (III)*

Atom	Distance (nm)	Symmetry
O-2	0.283	$(2-x, y-0.5, 1-z)$
O-5	0.284	$(1+x, y-1.0, z)$
O-1	0.304	$(1-x, y-0.5, 1-z)$
O-4	0.290	$(1-x, y-0.5, 1-z)$
O-8	0.287	$(1+x, y, z)$
O-5	0.284	$(1-x, y-0.5, 1-z)$
O-1	0.258	$(1+x, y, z)$
O-6	0.275	$(2-x, y-0.5, 1-z)$
O-8	0.297	$(2+x, y, z)$

Discussion

The present analysis of three compounds has allowed the detailed comparison of the phosphoglycerate analogues. The principal findings relate to the state of ionization of these compounds and their molecular conformations.

In compounds (I)

and (II)

the charge distribution is very similar. The carboxy groups are ionized, the amino group is protonated, and the arsonate group in compound (I) and the phosphonate group in compound (II) are ionized and carry a single delocalized negative charge.

In compound (III)

the carboxy and phosphate groups carry delocalized negative charges to balance the positive charge of the Ba²⁺ ion.

A comparison of the geometries around As and P atoms in the three crystal structures and in the highly accurately determined structure of the disodium salt of D-3-phosphoglycerate (IV) (Fewster & Fenn, 1982), summarized in Table 8, is consistent with the expected lengthening of the As-X bonds relative to the P-X bonds due to an increase of 0.010 nm in the covalent radius of the As atom

Fig. 4. Projection of the crystal structure of barium D-3-phosphoglycerate dihydrate (III) on the a - b plane, illustrating the packing of the molecules in the unit cell, the hydrogen-bonding and Ba²⁺ co-ordination

Table 8. *Comparison of the arsonate, phosphonate and phosphate substituents*

Compound (I), DL-2-amino-4-arsonobutanoic acid monohydrate; compound (II), DL-2-amino-4-phosphonobutanoic acid; compound (III), barium D-3-phosphoglycerate; compound (IV), disodium D-3-phosphoglycerate (Fewster & Fenn, 1982).

Compound (I)		Compound (II)		Compound (III)		Compound (IV)	
Bond length (nm)		Bond length (nm)		Bond length (nm)		Bond length (nm)	
As-O-1	1.728(5)	P-1-O-3	1.587(7)	P-1-O-3	1.571(15)	P-1-O-6	1.565(5)
As-O-2	1.682(5)	P-1-O-2	1.495(5)	P-1-O-1	1.505(14)	P-1-O-5	1.477(5)
As-O-3	1.667(5)	P-1-O-1	1.508(7)	P-1-O-2	1.499(13)	P-1-O-7	1.469(5)
As-O-4	1.926(5)	P-1-C-4	1.807(8)	P-1-O-4	1.590(15)	P-1-O-4	1.581(5)
Bond angle (°)		Bond angle (°)		Bond angle (°)		Bond angle (°)	
O-1-As-O-2	108.0(2)	O-1-P-1-O-2	109.5(4)	O-1-P-1-O-2	120.9(7)	O-5-P-1-O-7	118.2(3)
O-1-As-O-3	110.4(2)	O-1-P-1-O-3	107.4(4)	O-1-P-1-O-3	111.4(8)	O-7-P-1-O-6	115.5(3)
O-2-As-O-3	112.3(2)	O-2-P-1-O-3	115.7(4)	O-2-P-1-O-3	105.3(7)	O-5-P-1-O-6	105.8(3)
C-4-As-O-1	104.2(3)	C-4-P-1-O-1	106.3(4)	O-4-P-1-O-1	102.4(7)	O-4-P-1-O-5	104.2(3)
C-4-As-O-2	110.3(3)	C-4-P-1-O-2	109.1(4)	O-4-P-1-O-2	110.0(8)	O-4-P-1-O-7	110.5(3)
C-4-As-O-3	111.3(3)	C-4-P-1-O-3	108.4(4)	O-4-P-1-O-6	105.7(3)	O-4-P-1-O-6	105.7(3)
As-C-4-C-3	109.0(4)	P-1-C-4-C-3	111.5(5)	P-1-O-4-C-1	123.2(10)	P-1-O-4-C-3	123.2(4)

(Pauling, 1948). The arsonate and phosphonate groups have approximate tetrahedral geometries, with the largest O-X-O angle contained by the two O atoms carrying the delocalized negative charge.

The conformations of compounds (II), (III) and (IV) are very similar, but in compound (I), where C-2 and As are *trans* to each other, rotation about the C-2-C-3 bond results in a more extended conformation (Fig. 1). The As-N-1 bond distance is 0.494 nm in compound (I), the P-1-N-1 bond distance is 0.487 nm in compound (II) and the P-1-O-7 bond distance is 0.433 nm in compound (III) and 0.442 nm in compound (IV).

Although the conformations of the molecules analysed are influenced by intermolecular interactions in the hydrated crystal lattice, they are sufficiently similar to suggest that the arsonate is an effective analogue, on the basis of stereochemistry, of both phosphonate and phosphate.

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