Stereochemistry of Dihydrofolate Reductase Inhibitor Antitumor Agents: Molecular Structure of 'Baker's Antifol' (Triazinate) and 'Insoluble Baker's Antifol'

By Arthur Camerman and H. Warren Smith

Departments of Medicine and Pharmacology, University of Washington, Seattle, Washington 98195, *USA*

and Norman Camerman

Department of Biochemistry, University of Toronto, Toronto, Canada M5S 1A8

Abstract

The crystal and molecular structures of 4,6-diamino-1-[3-chloro-4-(m-dimethylcarbamoylbenzyloxy)phenyl]-1,2-dihydro-2,2-dimethyl-s-triazine ethanesulfonate (Baker's antifol, NSC 139105), and 4,6-diamino-1-[4-(4'-fluorosulfonyl-3'-methylanilinocarbonylethyl)-phenyl]-1,2-dihydro-2,2-dimethyl-s-triazine ethanesulfonate dihydrate (insoluble Baker's antifol, NSC 113423) have been determined by X-ray crystallography. Crystallographic data are: space group $P\bar{1}$, $a = 11·522$ (4), $b = 11·717$ (4), $c = 11·474$ (5) Å, $\alpha = 110·78$ (3), $\beta = 111·47$ (2), $\gamma = 87·84$ (3)°, $Z = 2$; and space group $P\bar{1}$, $a = 12·929$ (5), $b = 17·516$ (6), $c = 6·572$ (2) Å, $\alpha = 99·08$ (2), $\beta = 90·42$ (2), $\gamma = 94·08$ (2)°, $Z = 2$, respectively. The phase problem was solved, in each case by a multisolution method, and the structures were refined by least-squares techniques to yield $R = 0·060$ for the 4980 observed data for Baker's antifol and $R = 0·052$ for the 4345 observed data for insoluble Baker's antifol. These compounds are, respectively, reversible and irreversible inhibitors of dihydrofolate reductase and show clinical promise for use in cancer chemotherapy. Both molecules adopt an extended conformation and are protonated at one of the triazine ring nitrogens.

Introduction

Tetrahydrofolic acid (FAH_4) and dihydrofolic acid (FAH_2), enzymatic reduction products of the B vitamin folic acid, are essential for purine and pyrimidine biosynthesis. In the reaction whereby deoxyuridylate is converted to thymidylate by thymidylate synthetase, FAH_4 is oxidized to FAH_2 and must then be re-reduced by dihydrofolate reductase (DHFR) if pyrimidine biosynthesis is to continue. Inhibition of either thymidylate synthetase or DHFR can lead to cellular deficiency of thymidylate and 'thymine-less' cell death. If

very selective inhibitors could be developed, which could exploit differences between enzymes of normal and tumor cells and bind only to tumor enzymes, these agents would be extremely valuable cancer chemotherapy drugs.

Much effort has been expended on obtaining possible selective DHFR inhibitors, which could be used in cancer treatment, particularly by the late B. R. Baker, in whose laboratory scores of compounds have been designed, synthesized and evaluated for *in vitro* and *in vivo* antitumor activity (Baker, 1969). The pioneering work of Baker and his co-workers has resulted in the development of a number of reversible and irreversible DHFR inhibitors, which have shown activities against Walker 256 and Dunning leukemia ascites in the rat (Baker, Vermeulen, Ashton & Ryan, 1970; Baker & Ashton, 1973). Systematic design of new antifolate compounds has been hampered, however, by a lack of information on the stereochemistry of folic acid and of the inhibitors of DHFR. We have therefore undertaken a program of X-ray crystallographic analyses of folates and antifolates in order to supply conformational data, which can aid in the design and synthesis of new possible anticancer drugs.

Overall the most active of Baker's compounds are 4,6-diamino-1-[3-chloro-4-(m-dimethylcarbamoylbenzyloxy)]phenyl-1,2-dihydro-2,2-dimethyl-s-triazine ethanesulfonate (Baker's antifol), and 4,6-diamino-1-[4-(4'-fluorosulfonyl-3'-methylanilinocarbonylethyl)-phenyl]-1,2-dihydro-2,2-dimethyl-s-triazine ethanesulfonate dihydrate (insoluble Baker's antifol), which currently show promise in clinical trials as cancer chemotherapeutic drugs (McCreary *et al.*, 1977; Rodriguez *et al.*, 1977). It is these compounds whose crystal and molecular structures we now report. They are the first antifols which have been used clinically in cancer chemotherapy to be crystallographically investigated. They are also the first antifolate compounds of a size comparable to folic acid to have their conformations determined, and thus may provide indirect evidence for the structures of folates and

dihydrofolates. A preliminary report of this work has been published (Camerman, Smith & Camerman, 1978).

Experimental

Colorless crystals of Baker's antifol (BAF) were obtained from slow evaporation of an ethanol–2-propanol–water solution. Crystal data are presented in Table 1. Three reflections, monitored periodically, showed a 2% decline in intensity during the period of data collection. A linear decomposition function was used to calculate scale factors as a function of the serial order of collection. The standard geometrical corrections were applied to the intensity data, but no extinction, dispersion or absorption ($\mu = 0.27$ mm^{-1}) corrections were applied to the data set. Normalized structure amplitudes $|E|$ were obtained using the Wilson plot method.

The structure was solved using the multiple-solution tangent-formula program *MULTAN* (Germain, Main & Woolfson, 1971) and utilizing the 320 normalized structure factors with $|E| > 1.77$. Three origin-specifying reflections and eight additional reflections were used to compute 64 sets of phases by application of the Sayre relationship. The E map calculated from the phase set with the highest figure of merit (1.07) and the lowest residual (17.9%) revealed 33 of the 36 non-hydrogen atoms. When the three additional non-hydrogen atoms located on a difference map were added to the model, the discrepancy factor, $R = \sum ||F_o| - |F_c||/\sum |F_o|$, was 0.42.

The atomic positional and anisotropic thermal parameters were refined by block-diagonal least-squares procedures, where the function minimized was $w(|F_o|$

$- |F_c|)^2$. Unit weights were initially chosen, but for the final refinement statistical weights, $w = 1/\sigma_F^2$, were used. Atomic scattering factors for H (Stewart, Davidson & Simpson, 1965) and other atoms (Cromer & Mann, 1968) were taken from the literature. Computations were performed with the XRAY system (Stewart, 1976).

A difference-Fourier map, computed after several cycles of least-squares refinement, showed the positions of all 31 H atoms. A final cycle of full-matrix least-squares refinement of all atom positions with anisotropic temperature parameters for non-hydrogen atoms and fixed isotropic thermal parameters for the H atoms resulted in a discrepancy index $R = 0.060$ for 4980 reflections with $I > 2\sigma(I)$ and $R = 0.070$ for all 6141 reflections. A final difference-Fourier map showed three small peaks in the vicinity of the ethylsulfonate O atoms, which suggests that in 10 to 20% of the ions

Table 1. *Crystallographic data*

	BAF	IBAF
Molecular formula	$C_{21}H_{26}ClN_6O_2^+$. $C_2H_5O_3S^-$	$C_{21}H_{26}FN_6O_3S^+$. $C_2H_5O_3S^-.2H_2O$
Molecular weight	539.1	606.7
Crystal size	$0.24 \times 0.43 \times 0.93$ mm	$0.16 \times 0.6 \times 0.6$ mm
Space group	$P\bar{1}$ (No. 2)	$P\bar{1}$ (No. 2)
a	11.522 (4) Å	12.929 (5) Å
b	11.717 (4)	17.516 (6)
c	11.474 (5)	6.572 (2)
α	110.78 (3)°	99.08 (2)°
β	111.47 (2)	90.42 (2)
γ	87.84 (3)	94.08 (2)
Z	2	2
ρ_{calc}	1.336 Mg m^{-3}	1.375 Mg m^{-3}
Source of data	Picker FACS-II diffractometer	
Radiation	Mo $K\alpha$ (Nb-filtered); $\lambda = 0.71069$ Å	
Data-collection technique	θ–2θ scan	
Maximum sin θ/λ	0.650 Å^{-1}	0.595 Å^{-1}
Number of independent reflections	6141	5147
Number with $I > 2\sigma(I)$	4980	4345

Table 2. *Final fractional coordinates for BAF* ($\times 10^5$ *for* Cl *and* S; $\times 10^4$ *for* O, N *and* C)

Here and throughout this paper, the quantities in parentheses are the standard deviations in the units of the least significant digit quoted.

	x	y	z
Cl	29587 (8)	34774 (8)	85886 (8)
S	6444 (6)	72355 (7)	89600 (8)
O(1)	1238 (1)	3183 (1)	5946 (2)
O(2)	−3643 (2)	2378 (2)	5770 (3)
O(3)	−488 (2)	6484 (2)	8008 (2)
O(4)	378 (3)	8448 (2)	9673 (3)
O(5)	1591 (2)	7323 (3)	8463 (3)
N(1)	8138 (2)	9437 (2)	9893 (2)
N(2)	7304 (2)	7439 (2)	8729 (2)
N(3)	6033 (2)	8972 (2)	9302 (2)
N(4)	4046 (2)	8400 (2)	8998 (2)
N(5)	5167 (1)	6897 (1)	8101 (2)
N(6)	−3214 (2)	722 (3)	6342 (3)
C(1)	7145 (2)	8614 (2)	9291 (2)
C(2)	6241 (2)	6588 (2)	7656 (2)
C(3)	5967 (2)	6788 (3)	6349 (3)
C(4)	6550 (2)	5299 (2)	7545 (3)
C(5)	5081 (2)	8076 (2)	8784 (2)
C(6)	4103 (2)	5981 (2)	7520 (2)
C(7)	4027 (2)	5274 (2)	8238 (2)
C(8)	3053 (2)	4355 (2)	7679 (2)
C(9)	2143 (2)	4123 (2)	6404 (2)
C(10)	2221 (2)	4848 (2)	5703 (2)
C(11)	3197 (2)	5781 (2)	6264 (2)
C(12)	279 (2)	2924 (2)	4635 (3)
C(13)	−640 (2)	1911 (2)	4410 (3)
C(14)	−784 (3)	787 (3)	3399 (3)
C(15)	−1626 (3)	−151 (3)	3187 (3)
C(16)	−2342 (3)	44 (3)	3956 (3)
C(17)	−2229 (2)	1172 (2)	4957 (3)
C(18)	−1367 (2)	2104 (2)	5170 (3)
C(19)	−3068 (3)	1468 (3)	5729 (3)
C(20)	−4127 (4)	936 (4)	6974 (4)
C(21)	−2416 (4)	−224 (4)	6549 (5)
C(22)	450 (5)	6609 (4)	10969 (4)
C(23)	1276 (3)	6588 (3)	10216 (3)

the three O atoms are rotated about the S—C bond. Atomic fractional coordinates are given in Table 2.*

Recrystallization of insoluble Baker's antifol (IBAF) from a hot water–dimethylformamide mixture yielded colorless triclinic crystals. Crystal data are given in Table 1. Intensity data were scaled to correct for a 2% decline in intensity of the monitored reflections. Although the linear absorption coefficient was small ($\mu = 0.24$ mm^{-1}), an empirical correction based on the variation of intensity of a reflection on the φ axis as a function of φ angle was applied, along with the geometrical corrections. The 320 normalized structure

---

* Lists of structure factors, anisotropic thermal parameters for non-hydrogen atoms, and hydrogen-atom coordinates and isotropic thermal parameters for both compounds have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34468 (51 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

factors with $|E| > 1.72$ were used to solve the structure by direct methods. Six reflections, three general and three specifying the origin, were used by the program *MULTAN* to produce eight sets of phases. 34 of the 40 non-hydrogen atoms were located in an E map, calculated from the phase set with a figure of merit of 1.15 and residual of 15.8%. One phase set with a higher figure of merit and lower residual contained only positive signs and was discarded. The remaining non-hydrogen atoms were located from a difference-Fourier map. With all 40 heavy atoms in the model, R was 0.26.

The structure of IBAF was refined by the procedures described above for BAF. The F atom was identified by the shorter S—F bond length as compared with the S—O bond lengths. The final cycle of full-matrix refinement led to $R = 0.052$ for 4345 reflections with $I > 2\sigma(I)$ and $R = 0.060$ for all 5147 measured data. Refined coordinates for the non-hydrogen atoms are listed in Table 3.*

Table 3. *Final fractional coordinates of IBAF* (×10^5 *for* S; ×10^4 *for* F, O, N *and* C)

	x	y	z
S(1)	-43284 (7)	34063 (5)	-31298 (13)
S(2)	-33197 (6)	80231 (4)	88170 (11)
F	-4240 (2)	2631 (1)	-2863 (4)
O(1)	-1333 (2)	4657 (1)	5474 (3)
O(2)	-5387 (2)	3621 (2)	-2966 (5)
O(3)	-3949 (3)	3539 (2)	-5118 (3)
O(4)	-2821 (2)	8688 (1)	8057 (3)
O(5)	-3080 (2)	7304 (1)	7615 (4)
O(6)	-3100 (2)	8072 (2)	10978 (3)
O(7)	-3354 (2)	9228 (1)	4382 (4)
O(8)	-2249 (2)	7120 (1)	3591 (3)
N(1)	1281 (2)	10341 (2)	18998 (4)
N(2)	1802 (2)	9590 (2)	16046 (5)
N(3)	241 (2)	9292 (1)	17582 (3)
N(4)	-897 (2)	8379 (1)	15804 (4)
N(5)	765 (2)	8489 (1)	14594 (3)
N(6)	-1943 (2)	5470 (1)	3467 (3)
C(1)	1117 (3)	9734 (2)	17542 (5)
C(2)	1846 (3)	8826 (2)	14883 (5)
C(3)	2344 (4)	8872 (3)	12856 (7)
C(4)	2446 (3)	8342 (3)	16158 (8)
C(5)	48 (2)	8722 (2)	15976 (4)
C(6)	505 (2)	7834 (2)	12979 (4)
C(7)	678 (3)	7091 (2)	13263 (4)
C(8)	380 (3)	6475 (2)	11756 (5)
C(9)	51 (3)	7951 (2)	11210 (5)
C(10)	-263 (3)	7329 (2)	9705 (5)
C(11)	-110 (2)	6578 (2)	9954 (4)
C(12)	-453 (3)	5895 (2)	8390 (5)
C(13)	-1081 (3)	6016 (2)	6653 (5)
C(14)	-1449 (2)	5311 (2)	5176 (4)
C(15)	-2470 (2)	4961 (1)	1894 (4)
C(16)	-2504 (2)	4159 (2)	1744 (4)
C(17)	-3072 (2)	3711 (2)	162 (5)
C(18)	-2995 (2)	5283 (1)	426 (4)
C(19)	-3568 (2)	4850 (2)	-1168 (4)
C(20)	-4122 (3)	5253 (2)	-2656 (5)
C(21)	-3603 (2)	4043 (2)	-1260 (4)
C(22)	-4661 (3)	8101 (2)	8507 (5)
C(23)	-5022 (3)	8835 (3)	9680 (7)

Discussion

In their design of new antifolates, Baker and his co-workers have had to adhere to key structural principles. Steric considerations play a major role in antifolate drug-design strategy: successful inhibitors may utilize one part of their molecular architecture to bind to DHFR outside the enzyme active site and another part to block the active site from reaction with the folate substrate. Differences in molecular stereochemistry will also determine differential antifolate binding characteristics to the DHFR of normal and tumor cells. Folic acid was isolated and characterized about 30 years ago (Mowat *et al.*, 1948), but despite long-term efforts
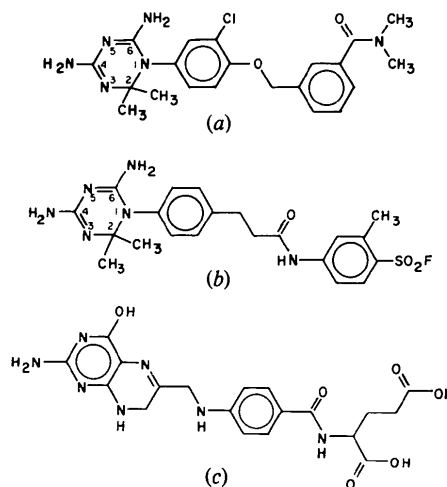
---

* See previous footnote.

Fig. 1. Structural formulas for (*a*) BAF, (*b*) IBAF and (*c*) dihydrofolic acid.

in many laboratories, it has never been successfully crystallized for an X-ray crystallographic structure determination. Likewise, the conformation of methotrexate, the most widely used antifolate cancer chemotherapy agent, has not yet been crystallographically elucidated. However, since BAF and IBAF (Fig. 1) are both of comparable size to folic acid and methotrexate, and have demonstrated clinical anticancer potential as inhibitors of dihydrofolate reductase, the three-dimensional conformations of these two 'nonclassical' antifols will enable us to draw strong inferences on the three-dimensional stereochemistry of folic acid and the classical antifols.

Bond distances and angles for BAF and IBAF are shown in Figs. 2 and 3, which also give the atom-numbering scheme. A significant feature of the three-dimensional structure of both molecules is their linearly extended conformation, as shown in Figs. 4 and 5. For BAF the relationship between the two substituted phenyl rings is described by the 176·4 (2)° phenyl—O—CH$_2$—phenyl torsion angle. In IBAF this relationship is described by three torsion angles, all close to 180°: phenyl—CH$_2$—CH$_2$—C −177·2 (3)°, CH$_2$—CH$_2$—C—N −173·5 (3)° and CH$_2$—C—N—phenyl −172·9 (3)°. In both molecules the triazine ring and the adjacent phenyl ring are nearly perpendicular; the angle between the planar portion of the triazine ring and the chlorophenyl ring is 78·9 (3)° for BAF, and 85·1 (4)° for the corresponding dihedral angle in IBAF. The two- or four-atom bridge between the phenyl groups is, in each case, approximately in the plane of the central phenyl ring. In the case of IBAF the terminal phenyl is also nearly in this plane with an angle between the two rings of 10·7 (4)°, while for BAF the terminal phenyl is rotated so that the dimethyl carbamoyl group is on the same side of the molecular
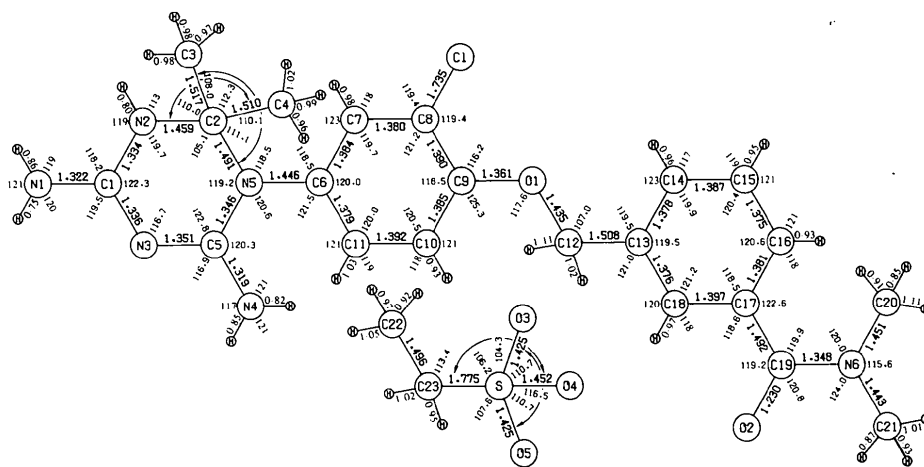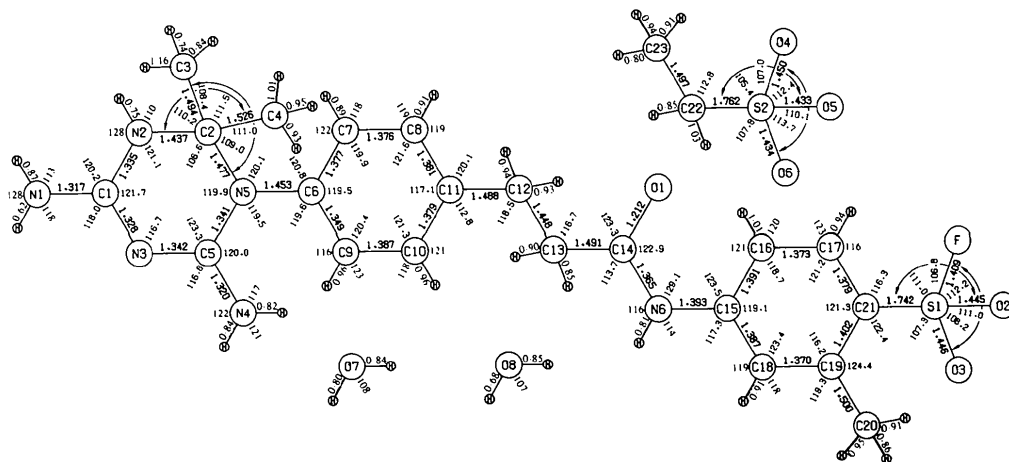

Fig. 2. Atomic nomenclature and bond lengths (Å) and interbond angles (°) for BAF. Estimated standard deviations are 0·002–0·007 Å for the heavy-atom bonds and 0·03 Å for bonds involving H atoms. Estimated standard deviations are 0·3° for heavy-atom angles and 1–3° for angles involving H atoms.

Fig. 3. Atomic nomenclature and bond lengths (Å) and interbond angles (°) for IBAF. Estimated standard deviations are 0·003–0·006 Å for the heavy-atom bonds and 0·03 Å for bonds involving H atoms. Estimated standard deviations are 0·3° for heavy-atom angles and 1–3° for angles involving H atoms.

long axis as the Cl atom. This results in an angle between the two phenyl rings for BAF of 66·6 (3)°. Protonation by the ethanesulfonic acid takes place at N(2) of the triazine ring for both molecules. A similar protonation situation was observed in the structure of the antifolate 2,4-diamino-6-benzyl-5-methylpyrido-[2,3-d]pyrimidine hydrobromide (Sternglanz & Bugg, 1973).

The hydrogen-bonding scheme employed in the BAF crystal utilizes all four amine H atoms plus the H on the ring N(2), to form an infinite ribbon along the a direction as shown in Fig. 6. One of these hydrogen bonds joins the bases about the center of symmetry at ½,1,1, while the others connect the cation to the O atoms of the sulfonate anion. In the IBAF crystal, the hydrogen-bonding pattern may be visualized as
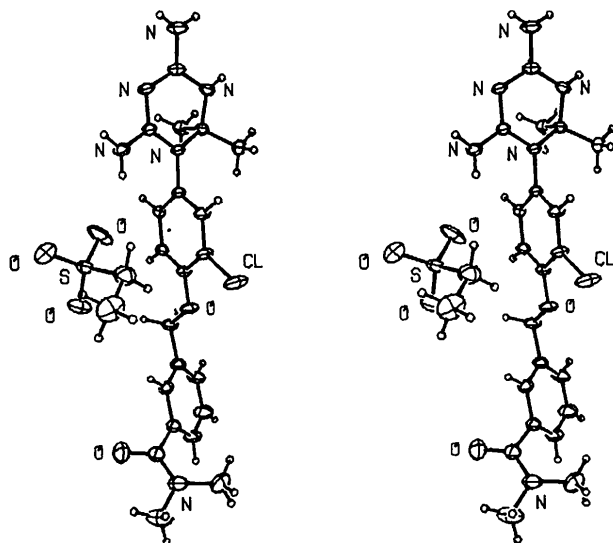

Fig. 4. Stereoscopic drawing of the protonated triazine cation and the ethylsulfonate anion in the BAF structure. The ellipsoid surfaces are drawn at the 50% probability level for all non-hydrogen atoms.

columns, extending along the c direction, of alternate ethanesulfonate ions and a pair of water molecules. As shown in Fig. 7, these columns are joined in pairs by the sulfonyl fluoride cations, each of which is attached by three hydrogen bonds to one column and by a single hydrogen bond to a centrosymmetrically related $EtSO_3^--H_2O$ column. As in BAF, one hydrogen bond in IBAF joins a pair of bases about a center of symmetry. The hydrogen-bonding parameters are given in Tables 4 and 5. The hydrogen-bonding pattern adopted by BAF is analogous to that observed in the structure of the pyrimidine hydrobromide antifolate mentioned above; in both structures all five H atoms bound to N form hydrogen bonds and one of these hydrogen bonds is between bases. In IBAF there are six H atoms bonded to N, of which five form hydrogen bonds and one of these is between bases. The structure of the antifolate trimethoprim (Koetzle & Williams, 1976) shows a somewhat different hydrogen-bonding scheme in the absence of a negative ion to act as an acceptor and in the absence of a protonated ring N. Hence hydrogen bonding in trimethoprim is limited to base-pairing. Trimethoprim and the benzylpyrido-pyrimidine compound are antibacterial antifolates but, unlike BAF and IBAF, they have not demonstrated potential use in cancer chemotherapy. The two antibacterial compounds are smaller molecules than BAF and IBAF, which are more comparable in size to folic acid and the clinically useful folic acid analog, methotrexate.

It is likely that both ends of the folic acid antagonists reported here would bind to DHFR. The triazine end, being similar to folic acid, likely effects the enzyme inhibition by blocking the active site, while the dimethylbenzamide end of BAF and the phenyl-sulfonyl fluoride end of IBAF probably interact with the enzyme outside the active site. Hence, the distances, shown in Table 6, between functional groups at the two ends of the antifolate will be a major factor in its
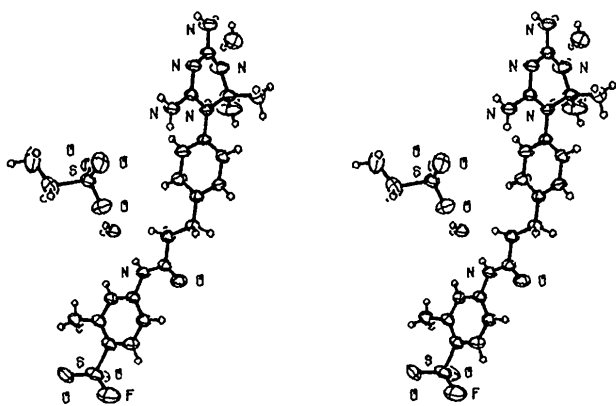

Fig. 5. Stereoscopic drawing of the protonated triazine cation, the ethylsulfonate anion and the two water molecules in the IBAF structure. The ellipsoid surfaces are drawn at the 50% probability level for all non-hydrogen atoms.
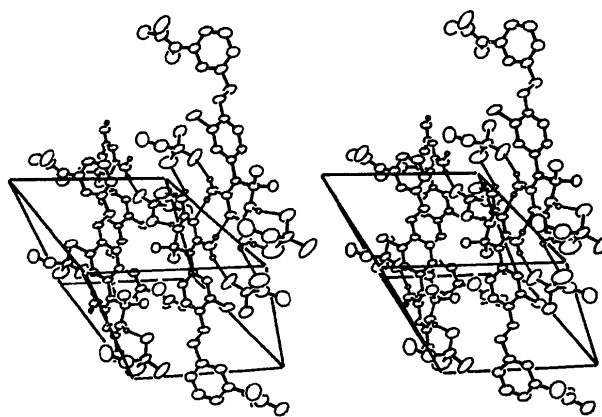

Fig. 6. Stereoscopic diagram of the BAF molecular packing. Only H atoms involved in hydrogen bonds are included. The origin is at the lower left-hand corner with a pointing away from the viewer, b up, and c to the right.

DHFR inhibitory power. This suggests a second binding site in DHFR 11–12 Å from the active site, with a possible additional site at a distance of 16–17 Å.

The linearly extended configuration as found for BAF and IBAF has also been suggested for methotrexate bound to DHFR (Matthews *et al.*, 1977). Although not all individual atoms can be discerned in the enzyme-inhibitor complex, there are strong indications that methotrexate is linearly extended with the pyrimidine end in a hydrophobic pocket, the *p*-aminobenzoyl moiety in a second hydrophobic pocket and the glutamic acid end bound at the enzyme surface.

Table 4. *Hydrogen-bond distances (Å) and angles (°) for BAF*

The e.s.d.'s for the three columns are 0·03 Å, 0·004 Å and 3° respectively.

	H···O(N)	N···O(N)	∠N–H···O(N)
N(1)–H(N1, 1)···O(4)^i	2·00	2·848	170
N(1)–H(N1,2)···O(4)^{ii}	2·45	2·851	115
N(2)–H(N2)···O(3)^i	2·23	3·013	166
N(4)–H(N4, 1)···O(5)	2·14	2·901	153
N(4)–H(N4, 2)···N(3)^{ii}	2·18	3·024	173

Symmetry code

(i) $1 + x, y, z$ (ii) $1 - x, 2 - y, 2 - z$

Table 5. *Hydrogen-bond distances (Å) and angles (°) for IBAF*

The e.s.d.'s for the three columns are 0·03 Å, 0·004 Å and 3° respectively.

	H···O(N)	O(N)···O(N)	∠O(N)–H···O(N)
N(4)–H(N4,1)···O(4)^i	2·16	2·943	158
N(4)–H(N4,2)···O(8)^i	2·16	2·904	146
N(2)–H(N2)···O(7)^{ii}	2·09	2·828	166
N(6)–H(N6)···O(8)	2·12	2·932	174
O(7)–H(O7,1)···O(4)	2·00	2·827	171
O(7)–H(O7,2)···O(6)^{iii}	2·03	2·808	166
O(8)–H(O8,1)···O(5)	2·04	2·841	157
O(8)–H(O8,2)···O(6)^{iii}	2·19	2·843	162
N(1)–H(N1,1)···N(3)^{iv}	2·17	3·021	166

Symmetry code

(i) $x, y, 1 + z$ (ii) $-x, 2 - y, 2 - z$
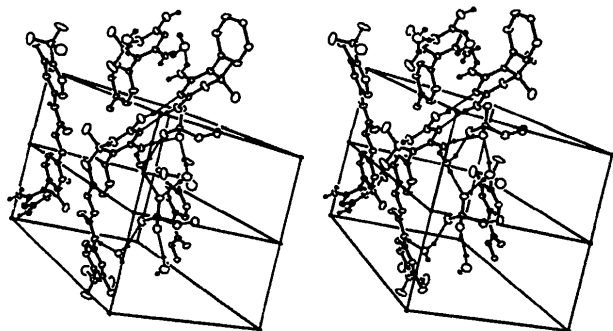(iii) $x, y, -1 + z$ (iv) $-x, 2 - y, 4 - z$

Fig. 7. Stereoscopic diagram of the IBAF molecular packing. Only H atoms involved in hydrogen bonds are included. The origin is at the lower left-hand corner with **a** to the left, **b** toward the viewer and **c** up.

Table 6. *Distances (Å) from triazine ring center*

	BAF	IBAF
Terminal phenyl-ring center	10·5	12·9
Carbonyl oxygen	11·9	9·9
Amide nitrogen	12·7	10·2
Sulfonyl oxygens		16·3–16·6
Sulfonyl fluorine		16·5

These results suggest that other antifolates and folic acid itself likely adopt a similar extended conformation.

References

BAKER, B. R. (1969). *Acc. Chem. Res.* **2**, 129–136.
BAKER, B. R. & ASHTON, W. T. (1973). *J. Med. Chem.* **16**, 209–214.
BAKER, B. R., VERMEULEN, N. M. J., ASHTON, W. T. & RYAN, A. J. (1970). *J. Med. Chem.* **13**, 1130–1140.
CAMERMAN, A., SMITH, H. W. & CAMERMAN, N. (1978). *Biochem. Biophys. Res. Commun.* **83**, 87–93.
CROMER, D. T. & MANN, J. B. (1968). *Acta Cryst.* B**26**, 321–324.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.
KOETZLE, T. F. & WILLIAMS, G. J. B. (1976). *J. Am. Chem. Soc.* **98**, 2074–2078.
McCREARY, R. H., MOERTEL, C. G., SCHUTT, A. J., O'CONNELL, M. J., HAHN, R. G., REITEMEIER, R. J., RUBIN, J. & FRYTAK, S. (1977). *Cancer (Philadelphia)*, **40**, 9–13.
MATTHEWS, D. A., ALDEN, R. A., BOLIN, J. T., FREER, S. T., HAMLIN, R., XUONG, N., KRAUT, J., POE, M., WILLIAMS, M. & HOOGSTEEN, K. (1977). *Science*, **197**, 452–455.
MOWAT, J. H., BOOTHE, J. H., HUTCHINGS, B. L., STOKSTAD, E. L. R., WALLER, C. W., ANGIER, R. B., SEMB, J., COSULICH, D. B. & SUBBAROW, Y. (1948). *J. Am. Chem. Soc.* **70**, 14–18.
RODRIGUEZ, V., RICHMAN, S. P., BENJAMIN, R. S., BURGESS, M. A., MURPHY, W. K., VALDIVIESO, M., BANNER, R. L., GUTTERMAN, J. U., BODEY, G. P. & FREIREICH, E. J. (1977). *Cancer Res.* **37**, 980–983.
STERNGLANZ, H. & BUGG, C. E. (1973). *Acta Cryst.* B**29**, 2191–2194.
STEWART, J. M. (1976). The XRAY system. Tech. Rep. TR-446. Computer Science Center, Univ. of Maryland, College Park, Maryland.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.