CHARVOLIN, J. & TARDIEU, A. (1978). *Solid State Physics*, Vol. 14, edited by F. SEITZ & D. TURNBULL, p. 209. New York: Academic Press.
EKWALL, P. (1975). *Advances in Liquid Crystals*, Vol. 1, edited by G. H. BROWN, ch. 1, p. 1. New York: Academic Press.
GUINIER, A. (1956). *Théorie et Technique de la Radiocristallographie*. Paris: Dunod.
HENDRIKX, Y. & CHARVOLIN, J. (1981). *J. Phys. (Paris)*, **42**, 1427–1440.
HENDRIKX, Y., CHARVOLIN, J., KÉKICHEFF, P. & ROTH, M. (1987). *Liq. Cryst.* **2**(5), 677–687.
KÉKICHEFF, P. & CABANE, B. (1987). *J. Phys. (Paris)*, **48**, 1571–1583.
KÉKICHEFF, P., CABANE, B. & RAWISO, M. (1984*a*). *J. Colloid Interface Sci.* **102**, 51–72.
KÉKICHEFF, P., CABANE, B. & RAWISO, M. (1984*b*). *J. Phys. (Paris) Lett.* **45**, L813–L821.
KÉKICHEFF, P., GRABIELLE-MADELMONT, C. & OLLIVON, M. (1988). *J. Colloid Interface Sci.* In the press.
LUZZATI, V. (1968). *Biological Membranes*, Vol. 1, edited by D. CHAPMAN, ch. 3, pp. 71–123. New York: Academic Press.
LUZZATI, V., TARDIEU, A. & GULIK-KRZYWICKI, T. (1968). *Nature (London)*, **217**, 1028–1030.
RANÇON, Y. & CHARVOLIN, J. (1987). *J. Phys. (Paris)*, **48**, 1067–1073.
RANÇON, Y. & CHARVOLIN, J. (1988). *J. Chem. Phys.* Submitted.
SADOC, J. F. & CHARVOLIN, J. (1986). *J. Phys. (Paris)*, **47**, 683–691.
SIEGEL, D. P. (1986). *Chem. Phys. Lipids*, **42**, 279–292.
WINSOR, P. A. (1968). *Chem. Rev.* **68**, 1–40.

Acta Cryst. (1988). B**44**, 406–412

Structural Studies of Analgesics and Their Interactions. XII. Structure and Interactions of Anti-inflammatory Fenamates. A Concerted Crystallographic and Theoretical Conformational Study

BY V. DHANARAJ AND M. VIJAYAN

Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, *India*

(*Received* 21 *September* 1987; *accepted* 25 *January* 1988)

Abstract

A theoretical conformational analysis of fenamates, which are *N*-arylated derivatives of anthranilic acid or 2-aminonicotinic acid with different substituents on the aryl (phenyl) group, is reported. The analysis of these analgesics, which are believed to act through the inhibition of prostaglandin biosynthesis, was carried out using semi-empirical potential functions. The results and available crystallographic observations have been critically examined in terms of their relevance to drug action. Crystallographic studies of these drugs and their complexes have revealed that the fenamate molecules share a striking invariant feature, namely, the six-membered ring bearing the carboxyl group is coplanar with the carboxyl group and the bridging imino group, the coplanarity being stabilized by resonance interactions and an internal hydrogen bond between the imino and carboxyl groups. The results of the theoretical analysis provide a conformational rationale for the observed invariant coplanarity. The second six-membered ring, which provides hydrophobicity in a substantial part of the molecule, has limited conformational flexibility in meclofenamic, mefenamic and flufenamic acids. Comparison of the conformational energy maps of these acids shows that they could all assume the same conformation when bound to the relevant enzyme. The present study provides a structural explanation for the difference in the activity of niflumic acid, which can assume a conformation in which the whole molecule is nearly planar. The main role of the carboxyl group appears to be to provide a site for intermolecular interactions in addition to helping in stabilizing the invariant coplanar feature and providing hydrophilicity at one end of the molecule. The fenamates thus provide a good example of conformation-dependent molecular asymmetry.

Introduction

Fenamates are a family of potent anti-inflammatory analgesics, prominent among them being meclofenamic, mefenamic, flufenamic and niflumic acids (Fig. 1). All of them, except niflumic acid (2-{[3-(trifluoromethyl)phenyl]amino}-3-pyridinecarboxylic acid), are *N*-aryl-substituted derivatives of anthranilic acid. The therapeutic activity of these analgesics is believed to be due to their ability to inhibit the biosynthesis of prostaglandins (Flower, 1974). In order to elucidate the mechanism by which they exert their influence on the enzymatic system, it is essential to have a thorough understanding of their molecular geometry, the interactions they are likely to be involved in, and the consequences of these interactions on their electronic structure and molecular geometry. An ongoing project in our laboratory, which was earlier concerned with anti-inflammatory pyrazole derivatives (Singh & Vijayan, 1974, 1977; Krishna Murthy, Vijayan & Brehm, 1979; Krishna Murthy & Vijayan, 1981*a*; Vijayan,

0108-7681/88/040406-07$03.00

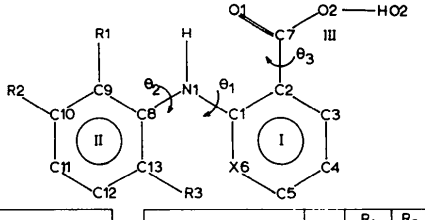
1983), is to derive this information through X-ray analysis of the crystals of fenamate molecules and their crystalline complexes with other suitable molecules. Detailed conformational computations employing semi-empirical potential functions have now been carried out on the members of the fenamate family in both ionized and neutral states. This paper presents a summary of the crystallographic observations and reports the results of the conformational calculations. These observations and results are then critically examined in terms of their relevance to drug action.

The fenamate molecules

Each fenamate molecule is essentially made up of three planar groupings: two six-membered rings, I and II, bridged by an imino N atom, and a carboxyl group, III, attached *ortho* to the imino N atom on ring I (Fig. 1). The differences between fenamates are due to different substituents on the second six-membered ring. A further difference between niflumic acid and flufenamic acid is the replacement of a CH group by an N atom in ring I of the former. Referring to Fig. 1, the conformation of each fenamate molecule can be described by three torsion angles, namely, θ_1 (C2–C1–N1–C8), θ_2 (C1–N1–C8–C9) and θ_3 (C1–C2–C7–O1).

Summary of crystallographic observations

The structures of mefenamic acid (McConnell & Company, 1976) and flufenamic acid (McConnell, 1973) were already available when these studies were started. The crystal structures of individual fenamates subsequently reported from this laboratory include those of meclofenamic acid (Krishna Murthy & Vijayan, 1981*b*), niflumic acid (Krishna Murthy & Vijayan, 1979) and a different form of flufenamic acid (Krishna Murthy, Bhat & Vijayan, 1982). Because they inhibit an enzyme system and are acidic in nature, initial efforts were made to prepare crystalline complexes of fenamates with basic amino acids and their



$\theta_1 = \tau_{C2-C1-N1-C8}$	$\theta_2 = \tau_{C1-N1-C8-C9}$	$\theta_3 = \tau_{C1-C2-C7-O1}$

Compound	X	R_1	R_2	R_3
Mefenamic acid	C	CH_3	CH_3	H
Meclofenamic acid	C	Cl	CH_3	Cl
Flufenamic acid	C	H	CF_3	H
Niflumic acid	N	H	CF_3	H

Fig. 1. The planar groupings in the fenamate molecule. The torsion angles that define the conformation and the numbering scheme are also indicated.

Table 1. *Torsion angles* (°) *in fenamate crystal structures*

Definitions of the torsion angles are given in Fig. 1.

	θ_1 C2–C1–N1–C8	θ_2 C1–N1–C8–C9	θ_3 C1–C2–C7–O1
Mefenamic acid	−179·3	−120·0	−1·7
Meclofenamic acid	−175·0	−87·0	−1·6
Niflumic acid	−174·0	5·0	−3·3
Flufenamic acid			
Form I	−179·0	−130·1	−3·7
Form II	−145·5	8·7	−7·6
Ethanolamine niflumate	−157·6	10·0	−10·8
Choline meclofenamate			
Molecule *A*	174·5	−111·0	−0·1
Molecule *B*	175·3	−109·4	−9·2
Ethanolamine meclofenamate			
Molecule *A*	174·7	−113·1	−9·7
Molecule *B*	174·6	−98·7	−0·5

Table 2. *Interplanar angles* (°) *in the crystal structures of fenamates and their complexes*

See text and Fig. 1 for the designation of the planar groupings.

	I and II	I and III	II and III
Mefenamic acid	62·4	2·4	60·5
Meclofenamic acid	80·2	1·3	81·4
Niflumic acid	8·7	2·4	10·2
Flufenamic acid			
Form I	52·8	3·8	51·9
Form II	43·0	6·7	45·4
Ethanolamine niflumate	32·2	12·9	32·6
Choline meclofenamate			
Molecule *A*	69·8	4·6	65·8
Molecule *B*	71·3	10·5	67·8
Ethanolamine meclofenamate			
Molecule *A*	65·4	11·5	56·0
Molecule *B*	77·6	5·3	72·9

derivatives. These efforts failed despite repeated trials under different conditions. The next best choice was to try complexation with ethanolamine and choline, since, apart from serving as model compounds for basic amino acids, they form part of the polar headgroups of membrane phospholipids. These experiments yielded good single crystals of (1) a hydrated 1 : 1 complex of niflumic acid and ethanolamine (ethanolamine niflumate), (2) a hydrated 1 : 1 complex of meclofenamic acid and choline (choline meclofenamate), and (3) a 1 : 1 complex between meclofenamic acid and ethanolamine (ethanolamine meclofenamate). The crystal structures of these complexes have been reported (Dhanaraj & Vijayan, 1983, 1987). In these complexes, the carboxyl groups are deprotonated and hence negatively charged.

The torsion angles θ_1, θ_2 and θ_3, and the interplanar angles between the three planar sections of each molecule (I, II and III) in the five crystal structures of neutral fenamates and the three complexes, are listed in Tables 1 and 2 respectively. The salient features observed in these eight structures are the following:

(1) The six-membered ring carrying the carboxyl group is coplanar with the carboxyl group and the imino N atom in all the structures. An internal N–H···O hydrogen bond with the imino N atom as donor and a carboxyl O atom as the acceptor exists in all of them. This hydrogen bond and the resonance

interactions between the groups concerned presumably stabilize the coplanar arrangement. The disposition of the second six-membered ring with respect to the coplanar arrangement is variable.

(2) Hydrogen-bonded and ionic interactions of the fenamate molecules almost exclusively involve the carboxyl group, as can be seen in the examples given in Fig. 2. The only case where an atom other than that belonging to the carboxyl group takes part in intermolecular interactions is in the structure of ethanolamine niflumate (Fig. 2*b*). In this structure, the hetero N atom in the first six-membered ring accepts a proton from a water molecule to form a hydrogen bond.

(3) A striking common feature of the fenamate structures is the segregation of hydrophobic and hydrophilic regions in the crystals. This feature is particularly evident in the structures in which the ethanolamine or the choline molecules and water molecules, when present, form hydrophilic columns. The bridging imino N atoms occur at the periphery of the columns. The hydrophilic columns are surrounded and separated by hydrophobic regions made up of the two aromatic six-membered rings and the substituents on the second ring. This mode of packing is presumably a consequence of the general chemical character of these amphiphathic molecules, with a small hydrophilic part made up of the carboxyl group and the bridging N atom and a large hydrophobic part made up of the two aromatic rings and the substituents on the second ring.

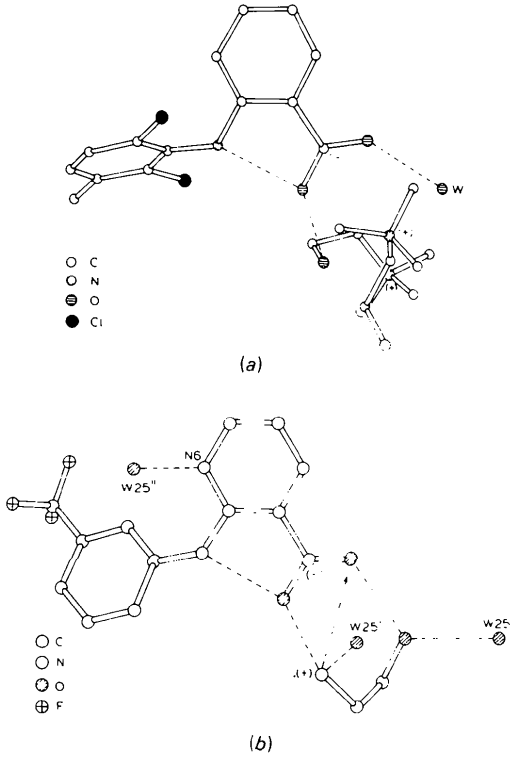


Fig. 2. The environment of (*a*) one of the meclofenamate anions in choline meclofenamate (Dhanaraj & Vijayan, 1987) and (*b*) the niflumate anion in ethanolamine niflumate (Dhanaraj & Vijayan, 1983).

Theoretical conformational analysis

Energy functions and choice of parameters

In semi-empirical methods based on classical potential-energy functions, the total potential energy of a system is partitioned into several discrete contributions:

$$E_{tot} = E_{nb} + E_{es} + E_{tor} + E_{hb} + E_{\theta} + E_{l}$$

where E_{nb} represents the non-bonded interaction energy, E_{es} the energy due to electrostatic interactions, E_{tor} the energy due to torsional strain, E_{hb} the energy due to the formation of hydrogen bonds, and E_{θ} and E_{l} the energies due to deformations in bond angle and bond length, respectively. In the present analysis, E_{θ} and E_{l} were neglected by keeping, for each molecule, the bond lengths and angles constant at the values obtained from crystal structure data. Hydrogen-bond energy was also not explicitly included in the calculations. A Buckingham or '6–exp' potential (Brant & Flory, 1965) was used to evaluate E_{nb}. The attractive term coefficients A_{ij} in this potential function were adapted from Hopfinger (1973) while the repulsive term coefficients B_{ij} were chosen such that E_{nb} for a pair of atoms i and j had a minimum value at an interatomic distance equal to the sum of the van der Waals radii of the atoms.

The partial charges on the atoms were obtained by adding the σ and the π charges determined using the Del Re (1958) and Huckel molecular orbital (HMO) (Pullman & Pullman, 1963) methods respectively. The parameters used for these calculations were obtained from Simon (1973).

As θ_1, θ_2 and θ_3 represent the three rotations about sp^2–sp^2 bonds, the periodicity, n, of the torsion function,

$$E_{tor} = V_{\theta}/2(1+\cos n\theta)$$

was given a value of 2. Through a careful examination of the appropriate bond length–bond order curves (Curl, 1959; Donohue, Lavine & Rollet, 1956; Pauling, 1960) and the values used by other workers in comparable situations, the barriers to rotation about θ_1, θ_2 and θ_3 were assigned values of 10, 5 and 5 kcal mol^{-1} respectively (1 kcal mol^{-1} $\equiv$ 4·1868 kJ mol^{-1}). Rotations about bonds are also possible in the case of substituents attached to the second six-membered ring, but they do not substantially affect the energy value and hence were ignored. These substituents were held fixed at energetically most favourable orientations determined by preliminary calculations on model systems. It was noted that the orientations of the methyl and trifluoromethyl groups derived from these model sys-

tems are in good agreement with those observed in the relevant crystal structures.

The bond lengths and angles of the anthranilic acid moiety used in the calculations were those averaged over the crystal structures of mefenamic, meclofenamic and flufenamic acids; those of the 2-aminonicotinic acid moiety were taken from the structure of niflumic acid. The corresponding dimensions for the meclofenamate and niflumate anions were derived from the structures of complexes containing them. No crystal structures involving mefenamate or flufenamate anions have been solved yet. Therefore, the anthranilate moiety in these ions was assumed to have the same dimensions as the anthranilic acid moiety in the free fenamates except in the carboxylate group which was assigned dimensions appropriate to the deprotonated species. Standard bond lengths and angles were used in the second six-membered ring and its substituents.

Computational strategy

Using the grid-search procedure, the potential energy of the molecule was evaluated at points taken at 10° intervals along θ_1, θ_2 and θ_3. The six-membered ring carrying the carboxylate group was fixed by specifying the atomic coordinates, and at each grid point, the rest of the molecule was generated using appropriate bond lengths and angles. For each conformation of the molecule, the non-bonded contact distances (1→4 and higher) were calculated. Conformations having contact distances less than the allowed 'extreme' limit (Hopfinger, 1973) for the appropriate atomic pair were eliminated. The total potential energy was evaluated for those conformations which have no such short contacts and the energy values were mapped out as a function of θ_1 and θ_2 at each fixed value of θ_3, thus generating two-dimensional maps in a form suitable for contouring. While contouring the maps, the minimum energy was taken to represent zero and successive contours were drawn at intervals of 1 kcal mol^{-1}.

Results

Calculations were performed on all fenamates in both of their ionization states. It was recognized and indeed verified that the energy values for the torsion angles θ_1, θ_2, θ_3 and $-\theta_1$, $-\theta_2$, $-\theta_3$ are almost the same. Therefore, while θ_1 and θ_2 were varied from 0 to 360°, θ_3 was varied only in the negative direction. The energy distribution remained substantially the same in each case when θ_3 was varied by about 20° from 0°. Larger variations resulted in an overall increase in energy. Moreover, θ_3 does not vary by more than 10° from 0° in any of the crystal structures examined so far. Thus, although θ_3 was varied between 0 and −30° in the energy calculations, only the maps at $\theta_3 = 0°$ and $\theta_3 = -10°$ are of practical interest. Further, these two maps are so similar that it is sufficient to consider only one of these. Thus, only the energy maps at $\theta_3 = 0°$, shown in Fig. 3, are considered in the discussion.

The energy maps for the neutral fenamate molecules and those for the corresponding anions are remarkably similar. This was not entirely unexpected as the changes that accompany ionization are not such as to lead to conformational changes. Ionization obviously affects primarily the carboxyl O2 atom. O2 and C7—O2 point away from the second six-membered ring, a rotation of which primarily causes conformational differences. Therefore, the effect of O2 and hence that of the changes associated with it on conformation are not substantial. The changes in the partial charge on O1 as well as the changes in the length of the bond C7—O1 are rather small. Thus deprotonation does not lead to appreciable changes in the distribution of energies. Therefore, only the maps corresponding to the neutral molecules are shown in Fig. 3.

Discussion

Comparison between theoretical results and experimental observations

Ten observed geometrical descriptions of fenamate molecules (including both the ionization states) given in Table 1 are available for comparison with theoretical results. The observed conformations, without a single exception, correspond to the conformational energy minima. This is all the more remarkable as environmental effects have not been taken into consideration in the conformational calculations. The environment of the molecules in the crystal structures, however, varies very substantially. This is particularly so in the ionic complexes in which the fenamate ions interact strongly with other ions and, in two structures, with water molecules. The close agreement between the results of conformational calculations and crystallographic observations indicates that the geometries observed in the crystal structures are intrinsic to the molecules and are largely unaffected by crystal packing.

Invariant structural features

The torsion angle θ_3, which defines the orientation of the carboxyl group with respect to the six-membered ring bearing it, has energetically the most favourable value at about 0°. A lone pair on O1 then approximately points towards the bridging imino N atom. It is clear from the geometry of the molecule that θ_1 should be close to 180° for the formation of a hydrogen bond between the N atom and O1. Indeed, most of the prominent features in the energy map have a θ_1 value in the neighbourhood of 180° despite the omission of hydrogen-bond energy from the calculations. Admittedly, there are some allowed regions at other values of θ_1, particularly at about ±60°. However, they are far less extensive than those with θ_1 values close to 180°.

Furthermore, the regions with $\theta_1 = 180°$ are obviously likely to have still lower energies than indicated in the maps if the energy of the hydrogen bond is also considered. Thus, the conformational calculations provide an energetic rationale for the observed invariant features, namely, the coplanarity of the carboxyl group and the six-membered ring bearing it with the internal hydrogen bond. It has been found that the activity of the compounds can be removed by chemical modifications which destabilize the coplanar arrangement through loss of the internal hydrogen bond (Cushman & Cheung, 1976). Thus, the invariant structural features referred to above appear to be critical for the clinical activity of fenamates.

Orientation and role of the second ring

The variability in fenamate structures is mainly due to the different substituents on the second six-membered ring and its orientation with respect to the relatively rigid anthranilic (or 2-aminonicotinic) acid moiety. The two rings tend to be mutually perpendicular in order to avoid unfavourable steric contacts when bulky substituents are present at the *ortho* positions as in meclofenamic and mefenamic acids. In fact, the dominant feature in the conformational maps corresponding to these acids is a broad minimum-energy region around $\theta_2 = 90°$. The shape of the minima is nearly the same in the two cases though there are slight differences in detail. In flufenamic acid, a coplanar arrangement of the two rings is prevented by steric interactions between the H atoms at the respective *ortho* positions of the two rings. However, as the H atom is much smaller than the Cl atom or a methyl group, the range of θ_2 is broader than that accessible to mefenamic and meclofenamic acids, as can be seen from the corresponding energy maps.

The effect on conformation of the trifluoromethyl group at the *meta* position of flufenamic and niflumic acids is marginal from a steric point of view as it points away from the anthranilic (2-aminonicotinic) acid moiety. Niflumic acid differs from flufenamic acid only

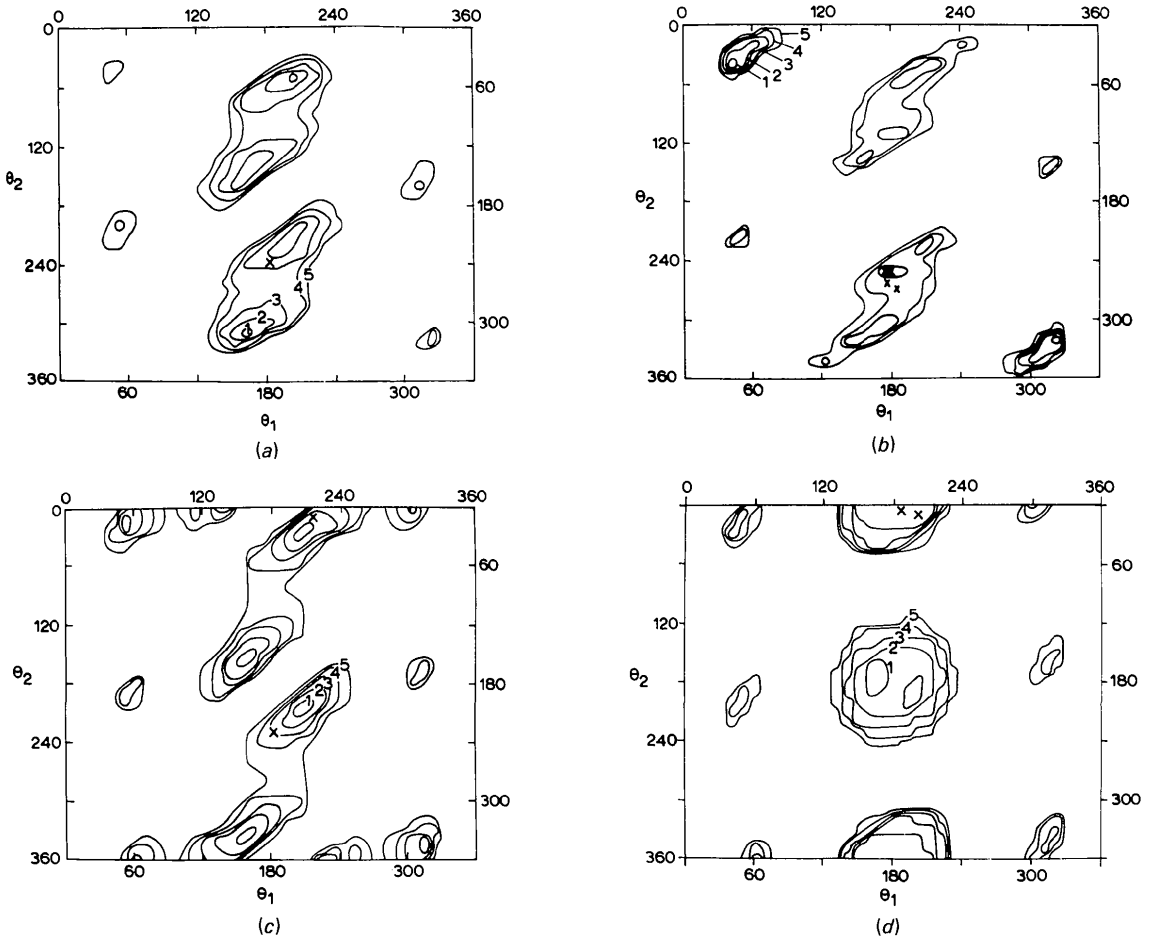


Fig. 3. Conformational energy maps at $\theta_3 = 0°$ of (*a*) mefenamic acid, (*b*) meclofenamic acid, (*c*) flufenamic acid and (*d*) niflumic acid. Contour levels (kcal mol^{-1}) are indicated. The observed conformations in appropriate crystal structures are indicated by crosses.

in the replacement of a CH group at position 6 of ring I in the latter by an N in the former. This, however, makes a substantial difference in the distribution of allowed regions in conformational space. The repulsive interactions between the H atoms at positions 6 and 9 (or 13), which prevent a coplanar arrangement of the two rings in flufenamic acid, are no longer relevant in niflumic acid. The energy map of niflumic acid is indeed dominated by two low-energy regions around $\theta_1 = 180°$, and $\theta_2 = 0$ and 180°, both corresponding to an almost coplanar arrangement.

The presence of the second six-membered ring is essential for the analgesic activity of fenamates as suggested by the fact that anthranilic acid by itself is pharmacologically inactive while *N*-phenylanthranilic acid and the fenamates derived from it are. One of the roles of this ring could be to endow hydrophobicity to a substantial part of the molecule. The common pattern of aggregation observed in the crystal structures containing fenamates appears to lend indirect support to this conclusion. In all these crystal structures the aggregation is such that the hydrophobic regions of different molecules, including the second ring, come together allowing the hydrophilic carboxyl group and other polar and water molecules, when present, to interact among themselves. Thus, it is conceivable that the binding site of the drug molecules contains hydrophilic and hydrophobic regions. The second six-membered ring could be important for interaction with the hydrophobic regions.

It has been noticed that the drugs become inactive when the second six-membered ring is replaced by alicyclic or aliphatic alternatives (Cushman & Cheung, 1976). Thus hydrophobicity is not a sufficient property of this part of the molecule for retention of activity. The aromaticity and the precise geometry of the molecule are also important. It would also appear that the binding site of the drugs has a fairly well-defined characteristic geometry, as the efficiency of the drugs is affected by substituents on the second ring. As mentioned earlier, the orientation of the second ring with respect to the rest of the molecule is somewhat variable and the question arises whether the binding site permits this variation. In this context, it is interesting to note that similarities exist between the conformational maps of the anthranilic acid derivatives. In fact, as illustrated in Fig. 4, there is a low-energy region and its inversion equivalent accessible to all three of them. This common region is contiguous to the main minimum region for each of the three compounds. Thus the energy required to rotate the second ring to the common region from any part of the main minimum region for any molecule is minimal. In view of the above, one is tempted to conclude that all the three molecules assume the same conformation, corresponding to the common minimum-energy region, when they bind to the appropriate enzyme in the prostaglandin synthetase system.

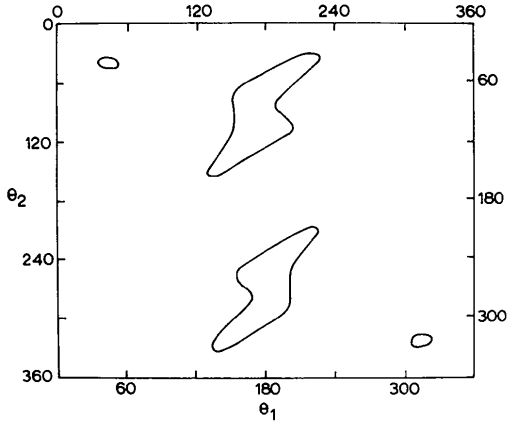


Fig. 4. Conformational map showing common minimum-energy regions of mefenamic, meclofenamic and flufenamic acids. The contour, drawn at 5 kcal mol^{-1}, encloses regions which are less than 5 kcal mol^{-1} in the energy maps of *all* the three molecules.

Structural distinctiveness of niflumic acid

It is interesting to note that the distribution of energetically favourable conformations of niflumic acid (Fig. 3) is substantially different from that of the other fenamates. Indeed, niflumic acid is the only fenamate which can, and does, adopt a near coplanar conformation. Furthermore, the common minimum-energy region for the other three fenamates lies just at the periphery of one of the minimum-energy regions for niflumic acid. Therefore, the conformation of niflumic acid in the enzyme–drug complex could well be different from that of the other three fenamates. Thus, the results of the theoretical calculations and crystallographic studies appear to provide a conformational rationale for the observation that the nature of inhibition of prostaglandin synthesis by niflumic acid is different from that of the other fenamates (Cushman & Cheung, 1976). Furthermore, niflumic acid has, in comparison with other fenamates, an additional site of interaction in the hetero N atom in the first six-membered ring. This may also contribute to the difference in the nature of inhibition by niflumic acid. Indeed the most significant result of this study is perhaps the identification of a common geometry accessible to fenamates based on an anthranilic acid moiety, and the differentiation of this class from niflumic acid, which is based on a 2-aminonicotinic acid moiety.

The role of the carboxyl group

All the fenamate molecules have two possible sites of interactions. They are the carboxyl group on the first six-membered ring and the bridging imino group. The geometry of the molecules, as elucidated through

conformational and X-ray studies, is such that the imino group is less readily accessible for intermolecular interactions than the carboxyl group. Indeed the former is not involved in intermolecular interactions in any of the relevant crystal structures whereas the latter is in all of them. Therefore, the carboxyl group is the only common site of specific interaction in all the fenamates. Thus, an important role of the carboxyl group would appear to be as a site for intermolecular interactions. As noted earlier, it also helps stabilize the invariant coplanar feature in the molecule, in addition to endowing hydrophilicity to one end of the molecule.

Molecular asymmetry

Seven out of the eight crystals containing fenamate molecules studied so far are centrosymmetric. In the eighth, there are two molecules in the asymmetric unit, related to each other by a pseudo-inversion centre. Thus in every crystal, half of the molecules are related to the other half by an inversion centre. This is hardly surprising since they do not contain chiral centres, nor do they have any obvious overall asymmetry. However, it was observed that the molecule and its inversion equivalent are non-superimposable. Thus, they are asymmetric and the crystals contain a *racemic* mixture. As can be seen from Table 1, for all practical purposes, the torsion angle θ_3 can be approximated to 0° and θ_1 to 180° in all fenamates. Thus the two isomers of a given fenamate molecule can be differentiated essentially by the sign of θ_2. In the conformational energy maps of all the fenamates except niflumic acid, the main allowed regions corresponding to the positive and negative values of θ_2 are separated by a disallowed region (energy greater than 5 kcal mol^{-1}). The strategy adopted in the conformational analysis does not permit the evaluation of the actual potential-energy barrier between the regions. Hence, it cannot yet be ascertained whether rapid interconversion between the two isomers is possible. Thus, the physiological role, if any, of the observed asymmetry needs further elucidation.

The authors thank the University Grants Commission, India, for support.

References

BRANT, D. A. & FLORY, P. J. (1965). *J. Am. Chem. Soc.* **87**, 663–664.

CURL, R. F. (1959). *J. Chem. Phys.* **30**, 1529–1536.

CUSHMAN, D. W. & CHEUNG, H. S. (1976). *Biochim. Biophys. Acta*, **424**, 449–459.

DEL RE, G. (1958). *J. Chem. Soc.* pp. 4031–4040.

DHANARAJ, V. & VIJAYAN, M. (1983). *Acta Cryst.* C**39**, 1398–1401.

DHANARAJ, V. & VIJAYAN, M. (1987). *Biochim. Biophys. Acta*, **924**, 135–146.

DONOHUE, J., LAVINE, L. R. & ROLLET, J. S. (1956). *Acta Cryst.* **9**, 655–662.

FLOWER, R. J. (1974). *Pharmacol. Rev.* **26**, 33–67.

HOPFINGER, A. J. (1973). In *Conformational Properties of Macromolecules*. New York: Academic Press.

KRISHNA MURTHY, H. M., BHAT, T. N. & VIJAYAN, M. (1982). *Acta Cryst.* B**38**, 315–317.

KRISHNA MURTHY, H. M. & VIJAYAN, M. (1979). *Acta Cryst.* B**35**, 262–263.

KRISHNA MURTHY, H. M. & VIJAYAN, M. (1981*a*). *Acta Cryst.* B**37**, 210–213.

KRISHNA MURTHY, H. M. & VIJAYAN, M. (1981*b*). *Acta Cryst.* B**37**, 1102–1105.

KRISHNA MURTHY, H. M., VIJAYAN, M. & BREHM, L. (1979). *Acta Cryst.* B**35**, 612–615.

MCCONNELL, J. F. (1973). *Cryst. Struct. Commun.* **2**, 459–461.

MCCONNELL, J. F. & COMPANY, F. Z. (1976). *Cryst. Struct. Commun.* **5**, 861–864.

PAULING, L. (1960). In *The Nature of the Chemical Bond*. Ithaca: Cornell Univ. Press.

PULLMAN, B. & PULLMAN, A. (1963). In *Quantum Biochemistry*. London: Interscience.

SIMON, Z. (1973). In *Quantum Biochemistry and Specific Interactions*. Tunbridge Wells, UK: Abacus Press.

SINGH, T. P. & VIJAYAN, M. (1974). *Acta Cryst.* B**30**, 557–562.

SINGH, T. P. & VIJAYAN, M. (1977). *J. Chem. Soc. Perkin Trans.* 2, pp. 693–699.

VIJAYAN, M. (1983). *Proc. Indian Acad. Sci. (Chem. Sci.)*, **92**, 463–471.

Acta Cryst. (1988). B**44**, 412–426

Static Deformation Densities for Cytosine and Adenine

BY MIRIAM EISENSTEIN

Department of Structural Chemistry, The Weizmann Institute of Science, Rehovot 76100, *Israel*

(*Received* 17 *September* 1987; *accepted* 29 *February* 1988)

Abstract

X-ray diffraction intensities for cytosine monohydrate have been measured at 97 K, to $2\sin\theta/\lambda = 2{\cdot}74$ Å^{-1}, and used in a deformation refinement. Crystal data for cytosine monohydrate at 97 K: $a = 7{\cdot}728\ (1)$, $b = 9{\cdot}817\ (3)$, $c = 7{\cdot}520\ (1)$, $\beta = 100{\cdot}50°$, $V = 560{\cdot}94$ Å^3, $R = 0{\cdot}0341$ for 6456 unique reflections. The experimental static deformation density of cytosine compares very well with the corresponding theoretical

0108-7681/88/040412-15$03.00