Acta Cryst. (1992). B48, 471–476

Configuration of Amidines and Factors Governing their Hydrogen-Bonding Patterns

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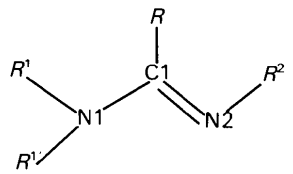
(*Received* 28 *February* 1991; *accepted* 9 *January* 1992)

Abstract

On the basis of 22 amidine structures the rules governing the amidine *cis–trans* configuration are developed. In amidines the most bulky groups bonded to N1, N2 or C1 tend always to be in the *trans* position and the molecular configuration depends on the relative sizes of these substituents. The configuration is also the cause of the different hydrogen-bonding patterns in secondary formamidines (*E,E* configuration, cyclic dimers) and all other secondary amidines (*E,Z* configuration, infinite chains bonded by N—H···N hydrogen bonds or linear dimers).

Introduction

Amidines are biologically active compounds containing the following group:



Structural studies on amidine systems have been carried out in this laboratory with the aim of tracing the changes induced in the geometry of the amidine group by different substituents and the factors which govern the *cis–trans* isomerism and hydrogen *h* bond formation. Our task was to investigate how the disposition of the side groups relative to the central amidine group depends on the character and dimensions of these groups, and to determine the extent of equalization of the C1—N bonds, the degree of conjugation between the amidine skeleton and substituents, and the overall configuration and conformation of the molecules. The amidine group, like the amide group, tends to be planar owing to the partial double-bond character of the C1—N1 bond. The planarity of the substituted amidine core ensures optimal interaction between trigonally hybridized C and N atoms at an energy cost equal to that required to bring the bonds at N1 into the common plane. Bulky substituents at the amidine core introduce steric hindrance and, depending on the size of the substituent, can force the molecule to undergo geometrical and conformational changes in order to relieve the strain. On the other hand, they may produce a total decoupling between the amidine π-electrons and the $2p$ orbital of the amidine N atom.

Conjugation of the N—C—N group

Significant insight into the conjugation of the N—C—N group can be obtained from the mutual dependence of the bond lengths. As the heteroatoms in the amidine core are identical and are both sp^2 hybridized, one can choose the shorter of the two bonds as C=N. If in several molecules the conjugation is stronger or weaker due to steric effects, one can expect that the longer the C=N bond, the shorter is the C—N bond. This relationship may be disturbed by other factors, such as conjugation with the benzene ring, which can make the C=N bond longer without affecting C—N. On the other hand, conjugation with the *N*-phenyl substituent can weaken the N=C—N conjugation but need not disturb the bond-length dependence (Exner, Budesinsky, Hnyk, Vesetecka & Raczyńska, 1988).

Of course, on complexation or protonation these two bonds become equivalent with a high degree of delocalization, but in this paper we focus our attention on free unprotonated amidines.

The difference Δ between the C—N and C=N bond distances is an indication of the degree of delocalization around the N—C—N skeleton. We have compared this value for 12 amidine structures which have been solved in this laboratory and an additional 10 structures solved elsewhere (Table 1). In the secondary N^1,N^2-diphenylformamidine [Table 1, (*m*)] (Anulewicz, Krygowski & Pniewska, 1987) Δ is very small [0.009 (4) and 0.012 (4) Å in the two independent molecules]. In N^1,N^2-bis(*p*-chlorophenyl)formamidine (*s*) (Anulewicz, Krygowski & Pniewska, 1990) Δ is 0. In another formamidine, with different substituents at N1 and N2, N^1,N^1-(hexamethylene)-N^2-(*p*-nitrophenyl)formamidine (*l*) (Krajewski *et al.*, 1981) $\Delta = 0.032$ Å. In the tertiary nonsymmetrical formamidine N^1,N^1-dimethyl-N^2-(*p*-

0108-7681/92/040471-06$06.00

Table 1. *Bond-distance differences, π-bond orders, bond angles and conformations of amidines*

Ref.	Compound name	Δ (Å)	π-bond order p^2 p^1	N—C—N ( )	Conformation at N2—C	N1—C
(*a*)	N^2-(*m*-Chlorophenyl)-N^1,N^2-pentamethylenebenzamidine	0.075 (6)	0.90.5	118.4 (4)	*E*	-
(*b*)	N^2-(*p*-Methoxyphenyl)-N^1,N^1-pentamethylenebenzamidine	0.089 (5)	1.00.5	119.4 (4)	*E*	
(*c*)	N^1-Methyl-N^1-phenyl-N^2-(*p*-tolyl)benzamidine	0.079 (6)	1.00.5	119.0 (3)	*E*	*E*
(*d*)	N^2-(*p*-Bromophenyl)-N^1-methyl-N^1-(*p*-tolyl)acetamidine	0.093 (8)	1.00.5	118.0 (7)	*E*	*E*
(*e*)	N^2-(*p*-Nitrophenyl)benzamidine	0.075 (5)	1.00.6	125.9 (3)	*Z*	
(*f*)	N^1,N^2-Di(*p*-tolyl)acetamidine	0.081 (4)	1.00.5	120.7 (3)	*E*	*Z*
(*g*1)	N^1,N^1-Dimethyl-N^2-(*p*-nitrophenyl)formamidine	0.043 (4)	0.90.7	123.6 (3)	*E*	
		0.042 (4)		123.2 (3)		
(*g*2)	N^1,N^1-Dimethyl-N^2-(*p*-nitrophenyl)acetamidine	0.052 (6)	0.90.6	118.7 (3)	*E*	
		0.073 (6)		118.2 (3)		
(*g*3)	N^1,N^1-Dimethyl-N^2-(*p*-nitrophenyl)pivalamidine	0.087 (3)	0.90.4	124.2 (2)	*Z*	
(*h*)	N^2-(*p*-Bromophenyl)-N^1-methyl-N^1-phenylbenzamidine	0.078 (5)	0.90.5	118.9 (3)	*E*	*E*
(*i*)	N^1,N^1-(3-Oxapentamethylene)-N^2-phenylacetamidine	0.088 (2)	1.00.5	118.3 (1)	*E*	-
(*j*)	N^1-(*p*-Bromophenyl)-N^1-methyl-N^2-(*p*-bromophenyl)benzamidine	0.097 (11)	0.90.4	119.5 (6)	*E*	*E*
(*k*)	Acetamidine	0.046 (1)	0.90.6	125.5 (1)	*Z*	
(*l*)	N^1,N^1-(Hexamethylene)-N^2-(*p*-nitrophenyl)formamidine	0.032 (6)	0.90.7	122.1 (4)	*E*	
(*m*)	N^1,N^2-Diphenylformamidine	0.009 (3)	0.80.75	122.1 (3)	*E*	*E*
		0.012 (3)		123.0 (2)		
(*n*)	N^1,N^2-Diphenylbenzamidine	0.059 (8)	0.80.7	120.4 (5)	*E*	*Z*
		0.056 (7)	0.80.6	122.7 (5)		
(*o*)	N^2-(3-Chloromethyl)-1,2,4-thiadiazoleacetamidine	0.010 (5)	0.80.7	120.1 (3)	*Z*	
(*p*1)	N^1,N^1-(2,6-Dimethylpentamethylene)-N^2-phenylacetamidine	0.096 (3)	1.00.5	119.3 (2)	*E*	-
(*p*2)	N^1-(2,6-Dimethylpentamethylene)-N^2-phenylpivalamidine	0.162 (5)	1.10.2	126.9 (4)	*Z*	
		0.178 (5)	1.00.1	126.6 (3)		
(*q*)	N^1-(Diethylaminothiocarbonyl)benzamidine	0.056 (9)	1.00.7	123.4 (7)	*Z*	-
(*r*)	N^1-Methyl-N^2-phenyl-*p*-nitrobenzamidine	0.107 (4)	1.20.6	120.8 (3)	*E*	*Z*
(*s*)	N^1,N^2-Bis(*p*-chlorophenyl)-formamidine	0	0.80.8	121.0 (4)	*E*	*E*
		0		122.8 (4)		

References: (*a*) Tykarska, Jaskólski & Kosturkiewicz (1986*a*); (*b*) Tykarska, Jaskólski & Kosturkiewicz (1986*b*); (*c*) Oszczapowicz *et al.* (1986); (*d*) Ciszak *et al.* (1987); (*e*) Surma *et al.* (1988); (*f*) Ciszak *et al.* (1988); (*g*1), (*g*2), (*g*3) Ciszak *et al.* (1989); (*h*) Knychała *et al.* (1989); (*i*) Ciszak *et al.* (1990); (*j*) Tykarska & Kosturkiewicz (1991); (*k*) Norrestam *et al.* (1983); (*l*) Krajewski *et al.* (1981); (*m*) Anulewicz *et al.* (1987); (*n*) Alcock *et al.* (1988); (*o*) Ivasaki & Akiba (1981); (*p*1), (*p*2) Gilli & Bertolasi (1979); (*q*) Braun *et al.* (1988); (*r*) Tinant *et al.* (1989); (*s*) Anulewicz *et al.* (1990).

nitrophenyl)formamidine (*g*1) (Ciszak, Gdaniec, Jaskólski, Kosturkiewicz, Owsiański & Tykarska, 1989) $\Delta = 0.043$ (4) and 0.042 (4) Å in the two independent molecules, while in the secondary 'symmetrical' acetamidine, N^1,N^2-di(*p*-tolyl)-acetamidine (*f*) (Ciszak, Gdaniec, Jaskólski & Kosturkiewicz, 1988) $\Delta = 0.085$ (4) Å. In an amidine containing a large substituent, N^1,N^1-dimethyl-N^2-(*p*-nitrophenyl)pivalamidine (*g*3) (Ciszak *et al.*, 1989), $\Delta = 0.087$ (3) Å. The largest value of Δ [0.162 (4) and 0.178 (5) Å] was found for N^1,N^1-(2,6-dimethylpentamethylene)-N^2-phenylpivalamidine (*p*2) (Gilli & Bertolasi, 1979). A plot of the bond lengths, C=N *versus* C—N, (Fig. 1) shows a rough correlation with negative slope.

The bond lengths in the two compounds show some inconsistencies. We have calculated the bond orders from the semiempirical correlation function, $r = r_0 - 0.18p$, which relates the π-bond orders (p) to the π-bond distances (r), where r_0 is a standard single-bond distance [1.458 Å for the C—N bond, according to Norrestam, Mertz & Crossland (1983)]. The bond-order scheme with partially delocalized π-electrons is in agreement with the planar arrangement around C1 and N1.

In the majority of amidine structures, the sum of π-bond orders (Σp) is 1.4 to 1.6 (mean value 1.5), although there are two notable exceptions. In N^1,N^1-(2,6-dimethylpentamethylene)-N^2-phenylpivalamidine (*p*2) (Gilli & Bertolasi, 1979) $\Sigma p = 1.2$ to 1.3 which could be caused by the bulky substituents. The second case, N^1-methyl-N^2-phenyl-*p*-nitrobenzamidine (*r*) (Tinant, Dupont-Feneau, Declercq, Podlaha & Exner, 1989), for which the sum of π-bond orders equals 1.8, is difficult to explain. The C—N and C=N distances show a large Δ value and the C=N2 bond distance is extremely short [1.249 (4) Å] with an extended C1—C(*R*) bond [1.521 (4) Å]. In the remaining examples bond orders range from precisely or nearly equal in the case of formamidines [(*m*), (*s*)] (Anulewicz *et al.*, 1987, 1990) to typical values of 1.0 and 0.5 (*f*). We suspect that it is the presence of the H atom at C1 which is responsible

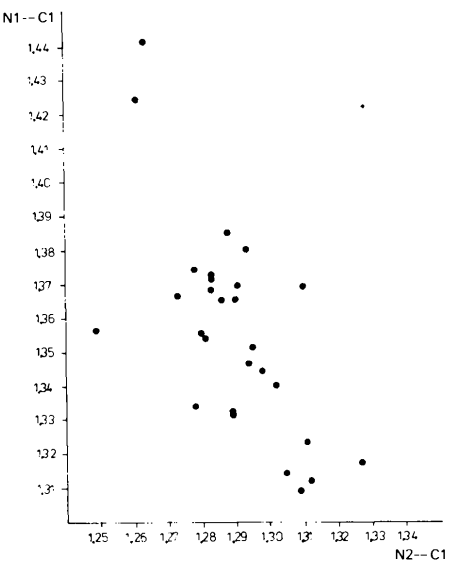


Fig. 1. The N2—C1 and N1—C1 bond lengths (Å) in amidines. (*a*) 1.290 (6), 1.365 (6); (*b*) 1.283 (5), 1.372 (5); (*c*) 1.286 (6), 1.365 (5); (*d*) 1.273 (8), 1.366 (8); (*e*) 1.280 (5), 1.3555; (*f*) 1.283 (4), 1.368 (4); (*g*1) 1.289 (4), 1.332 (4) and 1.289 (4), 1.331 (4); (*g*2) 1.294 (5), 1.346 (5) and 1.281 (5), 1.354 (5); (*g*3) 1.293 (3), 1.380 (3); (*h*) 1.291 (5), 1.369 (9); (*i*) 1.283 (2), 1.371 (2); (*j*) 1.288 (11), 1.385 (10); (*k*) 1.298 (1), 1.344 (1); (*l*) 1.302 (6), 1.334 (6); (*m*) 1.305 (3), 1.314 (3) and 1.311 (3), 1.323 (3); (*n*) 1.310 (8), 1.369 (8) and 1.295 (6), 1.351 (7); (*o*) 1.317 (5), 1.327 (5); (*p*1) 1.278 (3), 1.374 (3); (*p*2) 1.263 (5), 1.441 (5) and 1.262 (5), 1.424 (5); (*q*) 1.278 (9), 1.334 (10); (*r*) 1.249 (4), 1.356 (4); (*s*) 1.312 (4), 1.312 (4) and 1.309 (3), 1.309 (3) (references taken from Table 1).

for the equalization of the N1—C1 and C1—N2 bonds, while the symmetrical substitution at N1 and N2 as well as the presence of an H atom at N1 or N2 seems to play a minor role.

Orientation of substituents

The substituent at N2 can be positioned *trans* (*E*) or *cis* (*Z*) relative to N1. For nonsymmetrically substituted N1, two further isomeric forms (*E* and *Z*) can exist. According to the approach of Tinant *et al.* (1989), based on incomplete literature data 'The configuration is controlled by steric requirements of substituents on the second nitrogen atom. Disubstituted amidines apparently prefer configuration *E*'.

Among 12 structures described in our papers, two appear in the *cis* (*Z*) (C=N) configuration. (Table 1). The first, primary N^2-(*p*-nitrophenyl)benzamidine (*e*) (Surma, Jaskólski, Kosturkiewicz & Oszczapowicz, 1988), confirms the above rule, but the second, tertiary N^1,N^1-dimethyl-N^2-(*p*-nitrophenyl)-pivalamidine (*g*3) (Ciszak *et al.*, 1989) is in the *cis* configuration, in contrast to the predictions of Tinant, and in contrast to the analogous formamidine and acetamidine [(*g*1),(*g*2)] (Ciszak *et al.*, 1989). The *cis* configuration was also found in unsubstituted acetamidine (*k*) (Norrestam *et al.*, 1983), in the tertiary N^1,N^1-(2,6-dimethylpentamethylene)-N^2-phenylpivalamidine (*p*2) (Gilli & Bertolasi, 1979) and in the benzamidine derivative (*q*) (Braun, Richter, Sieler & Beyer, 1988). It thus becomes obvious that the amidine configuration does not only depend on the substituent at 'the second N atom'. It can be seen that the most bulky groups bonded to N1, N2 or C1 are always in the *trans* position. The molecular configuration depends on the relative size of these substituents.

N—C—N angle changes

The next problem concerns the widening or contracting of the N—C—N angle. An inspection of the amidine structures shows us that the N—C—N angle is wider in all *cis* (C=N) amidines than in the *trans* ones. A value of 118° is typical for the majority of *trans* (*E*) amidines substituted at C1. The situation observed in the case of the three N^1,N^1-dimethyl-N^2-(*p*-nitrophenyl)amidines [(*g*1),(*g*2),(*g*3)] (Ciszak *et al.*, 1989) can be expressed in the following way. The acetamidine derivative with a N—C—N angle of 118.7 (3) and 118.2 (3)° (in two independent molecules) belongs to the group of typical *trans* (*E*) (C=N) amidines. The widening of this angle in the formamidine (*E*) derivative to 123.6 (3) and 123.2 (3)° is due to the small volume of the H atom substituted at C1 and to the lack of repulsion from it. In the *cis* (*Z*) pivalamidine derivative (*g*3) the angle is 124.2 (2)°, similar to that in *cis* (*Z*) acetamidine [125.5 (1)°] (*k*) (Norrestam *et al.*, 1983) and in (*Z*) N^2-*p*-nitrobenzamidine [125.9 (3)°] (*e*) (Surma *et al.*, 1988).

As can be seen, in all *cis* (C=N) amidines the N—C—N angle is widened to values larger than 120°. The same situation is observed in *trans* formamidines. In *trans* amidines, with a substituent at C1, the angle ought to be smaller than 120°, because of steric hindrance. This rule does not work for amidines which are involved in hydrogen bonds.

Table 2. *Winkler–Dunitz parameters and the twist of phenyl rings relative the amidine skeleton in some amidines*

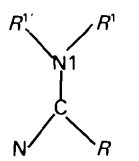
References are as given in Table 1.

Ref.	χ_C (°)	χ_N (°)	τ (°)	N2—S	N1—S	C1—S
(*a*)	−1.5	−11.3	8.4	59.7 (4)	–	60.2 (4)
(*b*)	2.0	27.5	17.4	54.6 (3)	·	61.6 (4)
(*c*)	1.9	9.4	12.8	70.2 (4)	63.1 (4)	60.0 (4)
(*d*)	1.1	13.7	22.7	90.1 (2)	67.0 (2)	·
(*e*)	0.2	8.3	3.7	78.9 (4)	·	21.2 (4)
(*f*)	·	·	·	88.1 (4)	39.4 (4)	–
(*g*1)	−2.4	8.3	−1.8	26.8 (4)	–	
	−2.8	5.9	−0.7	32.7 (4)	–	
(*g*2)	−2.7	0.8	2.4	61.3 (4)	–	
	3.2	−4.8	−4.5	70.7 (4)	·	·
(*g*3)	5.0	−10.6	33.2	59.2 (3)		
(*h*)	−1.7	10.9	−13.3	71.1 (5)	62.2 (5)	60.6 (5)
(*i*)	4.3	25.1	8.2	67.5 (1)	19.5 (1)	
(*j*)	1.1	13.7	22.7	66.4 (8)	63.7 (8)	47.2 (8)

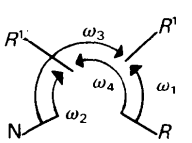
Out-of-plane and twist distortions

The sum of valence angles around N1 is near to 360° in the majority of the amidine molecules investigated. The Winkler & Dunitz (1971) parameters, which were introduced for the characterization of the out-of-plane and twist distortions of the amide group, may also be used to describe the amidine system (Table 2).

The parameters characterizing the out-of-plane deformations of the amidine group, χ_C and χ_N (see scheme below), indicate small deviations from planarity. There are some exceptions: (*g*3) (Ciszak *et al.*, 1989), an overcrowded molecule where χ_N equals −10.6°, and (*i*) with a morpholine ring. Molecule (*f*), which is involved in intramolecular hydrogen bonds and for which the donor hydrogen atom is used for the calculation of the Winkler–Dunitz parameters, cannot be considered as a typical case.



$\omega_1 = RCNR^1$
$\omega_2 = NCNR^{1'}$
$\omega_3 = NCNR^1$
$\omega_4 = RCNR^{1'}$



$\tau = (\omega_1 + \omega_2)/2$
$\chi_N = \omega_2 - \omega_3 + \pi = -\omega_1 + \omega_4 + \pi$
$\chi_C = \omega_1 - \omega_3 + \pi (\text{mod } 2\Pi)$

The values of τ show that the twist around the C1—N1 bond is minimal when the substituents are small, and it increases with increasing size of the substituents at C1. The largest τ value [33.2 (3)°] was found in the pivalamidine derivative mentioned above.

Of χ_C and χ_N, the χ_C parameter is always smaller, thus indicating that the sp^2 carbon shows stronger resistance to out-of-plane bending than the nitrogen atom.

Interaction between the N—C—N skeleton and ring substituents

The interaction between the N—C—N skeleton and the aromatic ring substituents may be rationalized from values of the cosine of the dihedral angle between the N—C—N skeleton and the aryl ring plane, since the superposition integral of the two p_π orbitals located on adjacent atoms is proportional to the cosine of the angle between them. In N^1,N^2-diphenylbenzamidine (*n*) (Alcock, Barker & Kilner, 1988) the torsion angles indicate a considerable interaction between the C1—N1 part of the skeleton and its associated phenyl ring, with a tendency towards planarity [−22.3 (7) and 15.5 (8)°].

The torsion angles between C=N2 and the phenyl skeletons [−75.5 (8) and 60.7 (7)°] show that some interaction of their π systems with the lone pairs of the sp^2-hybridized imino N atoms can take place. In the three N^1,N^1-dimethyl-N^2-(*p*-nitrophenyl)amidines (Table 2) [(*g*1),(*g*2),(*g*3)] (Ciszak *et al.*, 1989) the best planes between the amidine skeleton and the phenyl rings are 26.8 (4) and 32.7 (4)° in formamidine, 61.3 (4) and 70.7 (4)° in acetamidine, and 59.2 (3)° in pivalamidine. A considerable interaction between the amidine and phenyl fragments in formamidine seems to be possible as a result of the lack of steric hindrance. This hindrance, which is reduced by changing the configuration to *cis* (*Z*), is smaller in pivalamidine than in *trans* (*E*) acetamidine.

An angle of 90.1 (2)° between the phenyl ring and the amidine skeleton was found in N^2-(*p*-bromophenyl)-N^1-methyl-N^1-(*p*-tolyl)acetamidine (*d*) (Ciszak, Gdaniec & Kosturkiewicz, 1987), and a similar value [88.1 (4)°] occurs in N^1,N^2-di(*p*-tolyl)-acetamidine (*f*) (Ciszak *et al.*, 1988) where interaction of the phenyl π system with the lone pair of N2 is possible. As a rule, the angles between the phenyl groups and the amidine skeleton lie in the range 60–70°.

For example, in N^1-methyl-N^1-phenyl-N^2-(*p*-tolyl)benzamidine (*c*) (Oszczapowicz, Tykarska, Jaskólski & Kosturkiewicz, 1986) these angles are 63.1 (4), 60.0 (4) and 70.2 (4)°, and the phenyl rings are oriented like a fragment of a propeller screw (see Fig. 2). The twisting of the phenyl rings by about 60° seems to result from steric hindrance, which can be seen in space-filling models.

Results of hindered rotation about the N1—C1 bond

As rotation around the N1—C1 bond is hindered, in the case of a nonsymmetrically substituted N1, two further isomeric forms are possible, *E* and *Z*. In the above example the phenyl rings are situated on one side of the amidine core, while the other side is occupied by the methyl group alone. One can therefore conclude from the above example that the bulky methyl group can cause greater steric hindrance than the flat phenyl ring. The question arises then as to what is the sequence of substituents in order of size. We postulate the following sequence: H < phenyl < methyl < isopropyl < *tert*-butyl. The configurations of the C=N2 and C—N1 bonds are controlled by the steric interactions between these substituents.

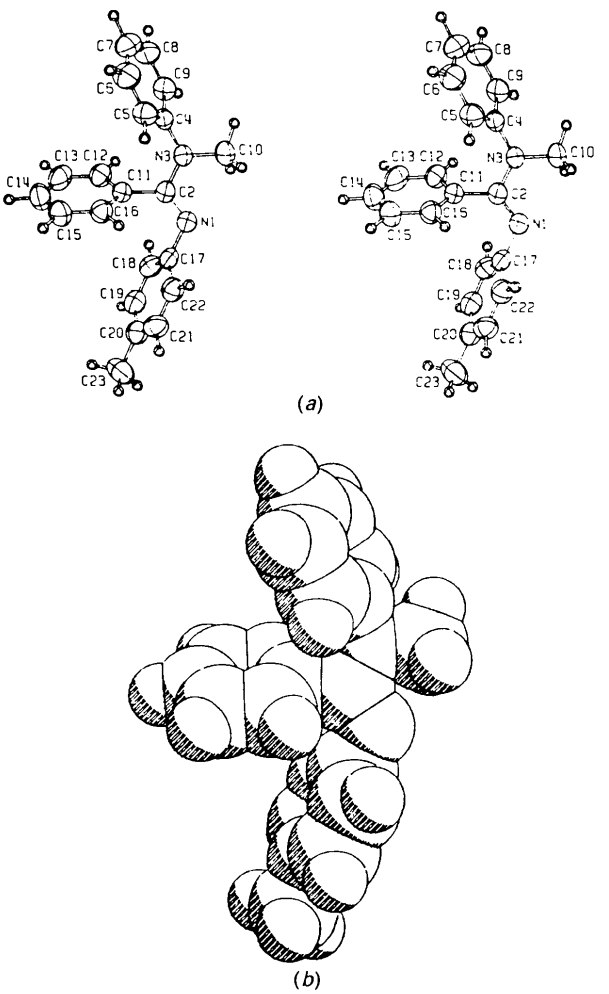
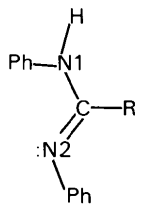


Fig. 2. N^1-Methyl-N^1-phenyl-N^2-(*p*-tolyl)benzamidine (*c*). (*a*) Stereoscopic view of the molecule. (*b*) Space-filling model of the molecule.

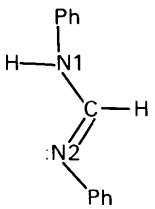
Intermolecular interactions

The secondary amidines, in which R^1 or $R^{1'}$ = H, are involved in N—H···N hydrogen bonds. We have solved one structure of a symmetrically substituted amidine, which could have two identical tautomeric forms (*f*) (Ciszak, Jaskólski & Kosturkiewicz, 1990). Alcock *et al.* (1981) solved a second structure of this kind (*n*). The molecular configuration relative to the C=N2 double bond is *trans* (*E*) and is *cis* (*Z*) relative to the C—N1 bond in both compounds [see (1) below]. The same configuration was found in N^1-methyl-N^2-phenyl-*p*-nitrobenzamidine (*r*) (Tinant *et al.*, 1989). This configuration is different from that in N^1,N^2-diphenylformamidine (*m*) (Anulewicz *et al.*, 1987), and N^1,N^2-bis(*p*-chlorophenyl)formamidine (*s*) (Anulewicz *et al.*, 1990) where a different hydrogen-bonding pattern is found [see (2) below]. The molecules of these formamidines form hydrogen-bonded cyclic dimers. Such a dimerization is possible owing to the electron lone pair at N2 and the N1—H donor group being located on the same side of the molecule.



(*E,Z*)

(1)



(*E,E*)

(2)

In N^1,N^2-di(*p*-tolyl)acetamidine (*f*) as well as in N^1,N^2-di(*p*-phenyl)benzamidine (*n*) (Alcock *et al.*, 1988) the lone pair at N2 and the N1—H donor are situated on opposite sides of the molecule, leading to different patterns of hydrogen bonds in the crystal. An intermolecular N—H···N hydrogen bond between the molecules links them so as to form chains in N^1,N^2-di(*p*-tolyl)acetamidine (*f*) and linear dimers in N^1,N^2-di(*p*-phenyl)benzamidine (*n*).

According to Sohar (1967), the IR spectrum of solid N^1,N^2-diphenylacetamidine indicates the formation of a cyclic hydrogen-bonded dimeric structure. Although we have not solved that particular structure, we would not expect this to be the case based on our present knowledge. We would suggest that only formamidines can form cyclic dimers linked by N—H···N bonds because the lone pair at N2 and N—H donor group are located on the same side of the molecule. In all other secondary amidines, the electron lone pair and donor group ought to be situated on opposite sides of the molecule due to steric interactions between the substituents.

Steric or chemical features?

The question arises as to whether it is true that steric factors, and not chemical features play the main role in the molecular configuration and conformation of amidines. We have solved two amidine structures which only differ in one respect. These are N^1-methyl-N^1-phenyl-N^2-(*p*-tolyl)benzamidine (*c*) (Oszczapowicz *et al.*, 1986) and N^2-(*p*-bromophenyl)-N^1-methyl-N^1-phenylbenzamidine (*h*) (Knychała, Rychlewska, Kosturkiewicz & Oszczapowicz, 1989). The difference is significant from a chemical point of view but minimal, as far as dimensions are concerned.

The crystals are isostructural, which suggests that a Br atom exerts the same steric effect as a methyl group, confirming the stereochemical and crystallochemical similarity between these different chemical groups. In both molecules the steric hindrance is possibly relaxed by twisting of the phenyl substituents at N1, C1 and N2 relative to the central amidine plane [63.1 (4), 60.0 (4) and 70.2 (4)° in the methyl derivative and 62.2 (5), 60.6 (5) and 71.1 (5)°, respectively, in the bromo derivative]. Thus we maintain that in amidines the disposition of the side fragments relative to the central amidine group depends more on the dimensions than on the chemical character of the substituents.

Concluding remarks

In amidines the extent to which the C1—N2 and C1—N1 bonds are equalized depends mainly on the substituent at C1. The presence of an H atom at C1 permits equalization of the bonds, while symmetrical substitution at N1 and N2 and the presence of an H atom at N1 seem to play a minor role.

In amidines the most bulky groups bonded to N1, N2 or C1 always tend to be in the *trans* position and the molecular configuration depends on the relative sizes of these substituents. This is also the reason for the different hydrogen-bonding patterns in secondary formamidines (*E,E* configuration, where the lone electron pair and N—H group are on the same side of the molecule) and all other secondary amidines (*E,Z* configuration, where the lone pair and N—H group are on opposite sides) with a substituent at C1 larger than an H atom.

The secondary amidine molecules in the *E,Z* configuration are able to form infinite chains linked by N—H···N hydrogen bonds or linear dimers with single hydrogen bonds, but not cyclic dimers.

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Structure of a Pepsin/Renin Inhibitor Complex Reveals a Novel Crystal Packing Induced by Minor Chemical Alterations in the Inhibitor

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(*Received* 23 *September* 1991; *accepted* 10 *February* 1992)

Abstract

The structure determination by molecular replacement methods of a monoclinic pepsin/renin inhibitor complex crystal, with two molecules in the asymmetric unit, is presented. The atomic model, consisting of two liganded pepsin molecules and 110 water molecules, has been refined to a final crystallographic R value of 0.139 for data between 8 and 2.9 Å resolution. The structure reveals a previously undescribed pepsin dimer formed predominantly by polar interactions. Inhibitor binding induces global structural changes in the native enzyme similar, but not identical, to the ones observed in other chemically similar pepsin/renin inhibitor complexes crystallized in an orthorhombic form. A region of the polypeptide chain (residues 292–297) which was not visible in the orthorhombic crystal is well ordered in the presently described structure; possibly induced by crystal contacts. The crystal packing of native pepsin is compared with the two different crystal forms of the inhibited enzyme.

Introduction

Pepsin belongs to the class of enzymes known as aspartic proteinases. Members of this class are widespread in nature and are responsible for a myriad of important commercial and biomedical processes (Kostka, 1985; Davies, 1990). Renin is a particularly interesting example of the group because of its role in the first, and limiting, step of the angiotensin–angiotensinogen cascade, which regulates hypertension in higher organisms. Highly purified renin from mammalian or recombinant sources has been difficult to obtain in large quantities and therefore porcine

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0108-7681/92/040476-13$06.00