NON-BONDED ATTRACTION AND THE CONFORMATION OF AROMATIC AMINO ACID DERIVATIVES[+]

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SUMMARY

NMR and X-ray crystallographic studies have shown that hydantoins derived from aromatic amino acids prefer folded conformation in solution because of non-bonded attraction between the π electrons and dipoles in the hydantoin ring. The favored conformation in the crystalline state may be different from that in solution. In a crystal, the effect of intramolecular non-bonded attraction may be counter-balanced by other factors, such as, lattice forces, hydrogen bonding and intermolecular interaction. Folded conformation is also found in cyclic dipeptides containing one or more aromatic amino acid residues. It is necessary to take into consideration non-bonded attraction as well as steric repulsion in studying the conformation of peptides and enzyme-substrate binding.

CONTENTS

[+]Dedicated to Professor R.B. Woodward on the happy occasion of his sixtieth birthday.

1. Introduction

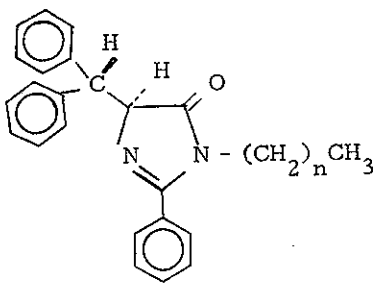
In recent years a growing body of evidence has been presented in support of significant interaction between polarizable π electrons and dipoles in the vicinity. The phenomenon of aromatic solvent induced shift ("ASIS") which found wide application in the early days of NMR spectroscopy is the result of such interaction at the intermolecular level.

The interaction between a dipole and the dipole induced by it in a π electron cloud constitutes a force of attraction. Such attraction at the intramolecular level if sufficiently strong, could substantially influence the conformation of small peptides and other polar compounds derived from aromatic amino acids. This type of nonbonded attraction could then be an important factor governing biological characteristics of these molecules.

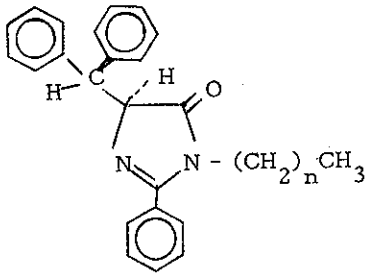
Since the beginning of the last decade several laboratories have studied the rotamer population of aromatic amino acid derivatives and found indications of nonbonded attraction between the aromatic ring and polar groups in another part of the molecule. We present here an overview of some of this work and give an account of the research in our own laboratory relating to this interesting phenomenon.

2. Studies on Labeled Hydantoins

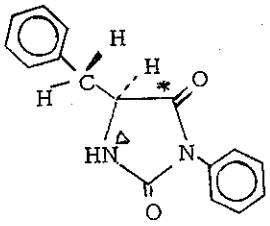
In 1967 we (I) made the fortuitous observation that the proton NMR spectrum of the azlactone analog (I) has some unusual features. The methyl signal appears at very high magnetic field (0.55 δ) at ambient temperature; raising the temperature of a solution of (I) in dimethyl sulfoxide shifts the methyl peak toward the more normal position at a lower field. These observations could be accounted for by assuming that favored conformation of (I) was such that the alkyl side chain was in the shielding cone of one of the phenyl groups. A to-scale model indicated that IB and IC satisfied this requirement. But the rotamers IB and IC are sterically hindered compared to IA. An attractive force between the aromatic rings and the strongly polar heterocyclic ring has to be assumed to provide a rationalization for favoring conformers IB and IC over IA.



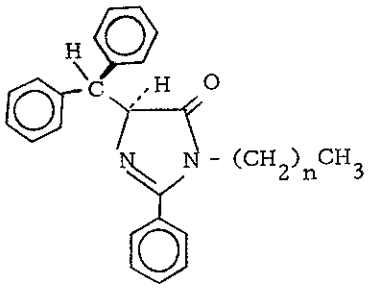
IA



IB



II



IC

*C = ^{12}C or ^{13}C

$^{\Delta}N$ = ^{15}N or ^{14}N

By coincidence, hydantoins (e.g. II) labeled with ^{13}C and ^{15}N were being prepared in our laboratory at this time (2). These heterocycles were to be used as intermediates for the preparation of labeled amino acids for biosynthetic studies. The availibility of labeled II prompted us to study their proton NMR spectra in detail (3).

The methylene protons in II are diastereotopic and anisochronous and are unequally coupled with the vicinal nuclei, namely, the proton at C-5, ^{13}C and ^{15}N (see Table 1).

Table 1

	H_a^*	H_b^*
J_{5H-H}, Hz	6.5 ± 0.1	4.4 ± 0.1
$J_{^{13}CH}$, Hz	2.7 ± 0.2	4.9 ± 0.2
$J_{^{15}NH}$, Hz	3.4 ± 0.2	3.0 ± 0.2

*H_a appears at higher field than H_b

The dependence of $J_{H-C-C-H}$ on the dihedral angle in accordance with the Karplus equation is well documented. In the absence of any indication to the contrary in the literature at that time, we assumed that a similar dependence would hold for $J_{^{13}C-C-C-H}$ and $J_{^{15}N-C-C-H}$. As a first approximation the Eq. (1) and (2) were used for calculations in this study.

$$J_{vic} = K_x \cos^2 \varphi \qquad \text{for } 0^\circ \leq \varphi \leq 90^\circ \quad (1)$$

$$= K_x' \cos^2 \varphi \qquad \text{for } 90^\circ \leq \varphi \leq 180^\circ \quad (2)$$

(K_x and K_x' are different for $J_{H-C-C-H}$, $J_{^{13}C-C-C-H}$ and $J_{^{15}N-C-C-H}$)

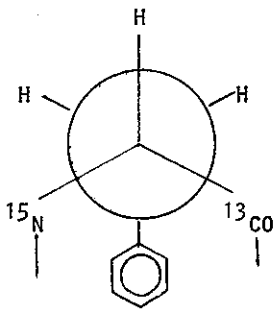
Under these conditions the relationship shown in Eq. (3) should hold as a first approximation for the *trans* coupling constants J_{Ht}, J_{Ct} and J_{Nt} (referring to $J_{H-C-C-H}$, $J_{^{13}C-C-C-H}$ and $J_{^{15}N-C-C-H}$, respectively).

$$J_{Ht} = n J_{Ct} = m J_{Nt} \quad (3)$$

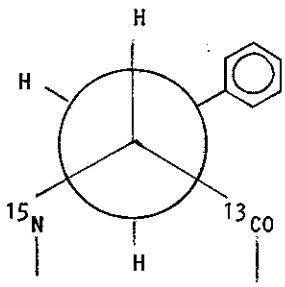
$$J_{Hg} = n J_{Cg} = m J_{Ng} \quad (4)$$

A similar relationship (Eq. (4)) should also be valid for the *gauche* coupling constants.

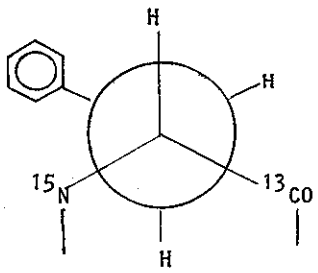
The Karplus equation predicts J_{Ht}/J_{Hg} to be in the neighborhood of 4. Assuming a specific value for J_t/J_g (between 3 and 5 in our calculations), it is possible to estimate the relative proportions of the three rotamers, IIA, IIB and IIC from the data in Table 1. Since it was not obvious as to which of the methylene protons, H_1 and H_2 in the Newman projections IIA, IIB and IIC would appear at higher field, two sets of calculations were made; in Case 1, H_1 is the proton H_b at lower field in Case 2, H_1 is the higher field proton H_a. The computed values are listed in Table 2.



IIA



IIB



IIC

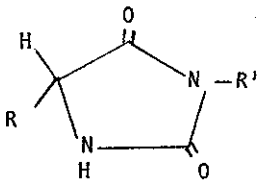
Table 2.

	Rotamer population, mole fraction			trans* couplings in Hz		
	IIA	IIB	IIC	H-C-C-H	^{13}C-C-C-H	^{15}N-C-C-H
Case 1	0.18±0.09	0.56±0.08	0.27±0.05	9.8±0.5	7.3±0.7	7.5±0.9
Case 2	0.42±0.14	0.16±0.06	0.42±0.08	12.3±0.8	7.7±1.2	5.8±0.8

*J_t/J_g is assumed to be 4; comparatively small changes in the rotamer population are caused when J_t/J_g is assumed to be 3 or 5.

On the basis of steric interaction which is generally synonymous with non-bonded repulsion, Case 1 would be preferred since the most sterically hindered conformer IIA is making the least contribution. However, we found evidence in support of Case 2 from a study of the pmr spectra of 3-ethylhydantoin (III) and 5-benzyl-3-ethylhydantoin (IV) (see Table 3). The methyl group in IV resonates at a higher field than in III - a result to be expected if the phenyl group in IV prefers a conformation which puts the N-Et group in its shielding cone. Such a conformation is compatible with Case 2 but not with Case 1. Also, the value of J(4) calculated for Case 2 (12.8 ± 0.8 Hz) is in better agreement with the literature (5) value of 13.5 Hz than the value (9.8 ± 0.5 Hz) calculated for Case 1. Therefore, we conclude that in II (and IV) the moderately hindered conformer IIB is making a minor contribution while IIA and IIC are making nearly equal and large contributions. This unusual rotamer population could be rationalized on the basis of non-bonded *attraction* between the aromatic ring and the positive end of the dipole (6) in the heterocyclic ring. While our work was in progress, Kopple and co-workers (7) reported proton NMR studies on diketopiperazines of type V which indicated that the arylmethyl side chain had the unexpected preferred conformation VA in which the aromatic ring faces the diketopiperazine ring. This preference for the crowded folded form was ascribed to a direct interaction between the two rings involving dipole-induced dipole interaction.

Table 3.



Solvent - DMSO

	Substituents	δ_{CH_3} (ppm from TMS) 40°	100°
III	R= H, R'=CH_2CH_3	1.08	1.08
IV	R=$C_6H_5CH_2$, R= CH_2CH_3	0.77	0.83

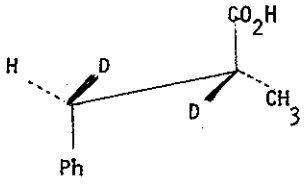
We presented our observations on the conformation of II at a meeting of the American Chemical Society in 1968 (8). The corresponding publication, however, was unacceptable to referees because of their doubts about the validity of Karplus type relationship for J_{vic} ^{13}C-H and J_{vic} ^{15}N-H.

The recent availability of ^{13}C -enriched compounds and pulsed NMR spectrometers has made possible in several instances to measure vicinal coupling between ^{13}C and ^{1}H. In 1972 Lemieux, Nagabhusan and Paul (9) reported evidence for the existence of a Karplus type relationship for $J_{^{13}C-C-C-^{1}H}$ similar to that for proton-proton vicinal coupling. Fieney, Hanson and Roberts (10) have demonstrated more recently that $J_{^{13}C-C-C-H}$ coupling constant between the α -CO_2 and the β -protons in amino acids can be used to deduce the side chain conformation of amino acids.

In 1968 Terui, Aono and Tori (11) demonstrated a geometrical dependence of the vicinal ^{14}N-H coupling in compounds. The N-C-C-H coupling is easier to measure when ^{15}N labeled compounds are used because ^{15}N has no quadruple moment. Lichter and Roberts (12) reported evidence for angular dependence of vicinal ^{15}N-H coupling

constants in amino acids.

In the light of these recent observations regarding Karplus type relationship, our approach to the determination of rotamer population by simultaneous consideration of vicinal H-H, ^{13}C-H and ^{15}N-H appears justified. It should be noted that the constants in the Karplus equation are sensitive to the environment of the nuclei under observation. The simplified, approximate form of the Karplus equation employed by us is convenient to use, but of necessity, the relative quantities of rotamers predicted would be only approximate in value. Nonetheless, this approach would provide a semi-quantitative measure of the relative importance of various rotamers.



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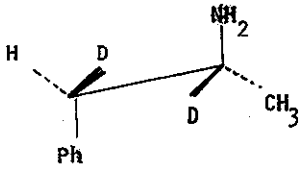
V



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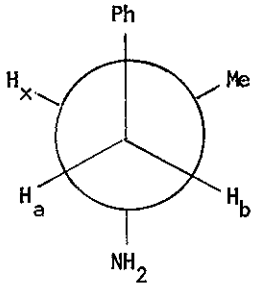
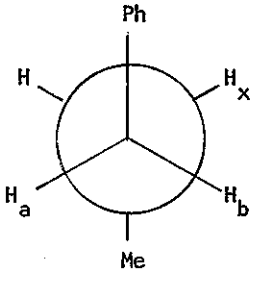
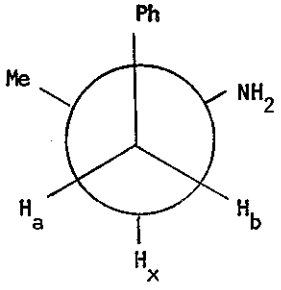
VI



VIA



VIB



VIC

Another approach for obtaining an approximate idea of the rotamer population is to assume reasonable values of J_{vic} trans and J_{vic} gauche and calculate the mole fractions of the rotamers A, B, and C that will satisfy the experimentally observed J_{H-H} or $J_{^{13}C-H}$ values. For calculations of this type it is necessary to identify the chemical shifts of H_a and H_b.

Bright, Platano and Jacobus (13) submitted 2-methylcinnamic acid of established E configuration to cis deuteration (D_2/Pd) and obtained a single product showing a single-proton resonance. This product (V) should then correspond to the enantiomers (2R, 3R)- and (2S, 3R)-2, 3-dideuterio-2-methyl cinnamic acid. Hoffmann rearrangement of the amide from this acid (V) would be expected to proceed with retention of configuration. The amphetamine so obtained was (1R, 2R)- and (1S, 2S)-1,2-dideuterio-1-phenyl-2-propylamine (VI) and the single-proton resonance at 2.69 corresponded to H_b. The H_a and H_c resonances were observed at 2.52 and 3.17, respectively for a sample of amphetamine and the ABX region of the spectrum led to the following values of coupling constants; J_{ab} = -14.29, J_{ax}=8.31, J_{bx}=5.84 Hz.

For determining the rotamer population, the following equations were used:

$$J_{ax} = n_a J_g + n_b J_t + n_c J_g$$

$$J_{bx} = n_a J_t + n_b J_g + n_c J_g$$

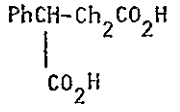
$$1 = n_a + n_b + n_c$$

These equations were solved by assuming J_t=13.6 and J_g=2.6 Hz and the following rotamer population was deduced:

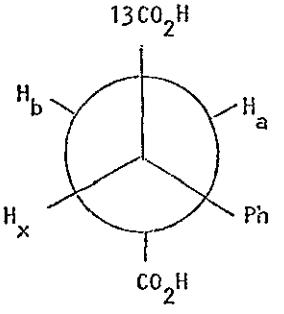
$$n_a = 0.29, \quad n_b = 0.52, \quad n_c = 0.19$$

It may be noted that the two most favored rotamers of amphetamines are those in which the amino group is gauche to the aromatic ring. The conformer with the highest population is (VI b) in which the methyl and the phenyl are trans to each other.

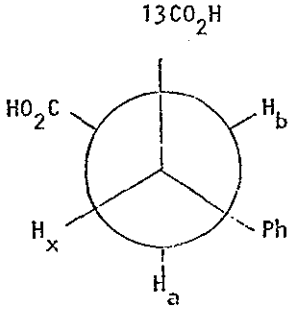
Rennekamp and Kingsbury (14) have studied the ^{13}C NMR spectrum of phenylsuccinic acid (VII) having about 60% enrichment of ^{13}C in one carbonyl group. Computer simulation of the spectra gave $J_{^{13}C-H}$ to be 4.3 and 2.5 Hz. Using the limiting values of J_t=13.5 Hz and J_g=2.8 Hz the relative abundance of the three conformers were calculated to be 0.58, 0.38 and 0.04. The label on the carbonyl group helped assign the chemical shifts of the proton H_a and H_b. It then became possible to deduce that VIIA was the most heavily populated conformer and the next heavily populated conformer was VIIB. This conformation was independent of ph, that is, unaffected by the formation of the mono- or diamion. On the other hand, in pyridine solution no ionization takes place but hydrogen bonding with the solvent occurs and the NMR parameters are changed. The conformer most favored in the solvent or acetone is VIIB.



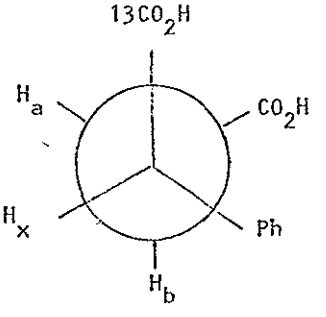
VII



VIIA



VIIB



VIIC

3. Proton NMR Studies

To obtain detailed information about possible non-bonded attraction in aromatic amino acid derivatives, a large number of hydantoin and thiohydantoin derivatives were prepared in our laboratory (15) from aliphatic and aromatic amino acids. A few representative examples of the hydantoins studied are shown in Table 4 and 5.

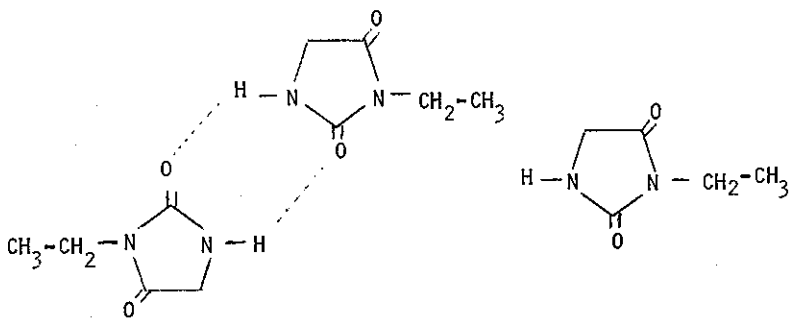
3.1 Proton Chemical Shifts of 3-Alkylhydantoins

In Table 4 are displayed the chemical shifts of the 3-ethyl protons of hydantoins and thiohydantoins derived from the α-amino derivative of acetic acid, phenylacetic acid, 3-phenylpropionic acid and 4-phenylbutyric acid. The upfield shift of the 3-alkyl proton signals demonstrates that the 5-benzyl-and 5-skatyl-hydantoins belong to a different category than the other hydantoins in Table 4.

The chemical shifts of the 3-alkyl protons are solvent dependent - they are more strongly influenced by a polar solvent such as DMSO-d_6 and CD_3COCD_3 than a less polar solvent ($CDCl_3$). The chemical shifts are independent of concentration variation when DMSO-d_6 is the solvent. In $CDCl_3$ solution, the N-H proton shifts upfield as the concentration decreases while the 3-alkyl protons remain virtually unchanged showing thereby that intramolecular forces have more effect on the 3-alkyl protons than intermolecular forces.

The proton NMR data on the compounds in Table 4 and other hydantoins led to the conclusion that in a strong solution in $CDCl_3$ there is intermolecular hydrogen bonding involving the -HN-CO- group (Figure 1). In DMSO-d_6 solution, there is participation by the solvent in hydrogen bond formation (Figure 2) leading to a strong dipolar pocket. The pronounced upfield shift of N-alkyl protons in DMSO-d_6 solution of benzyl and skatyl

FIGURE 1.



In Concentrated Solution

In Dilute Solution

FIGURE 2.

Hydrogen Bonding of Hydantoin in DMSO Solution.

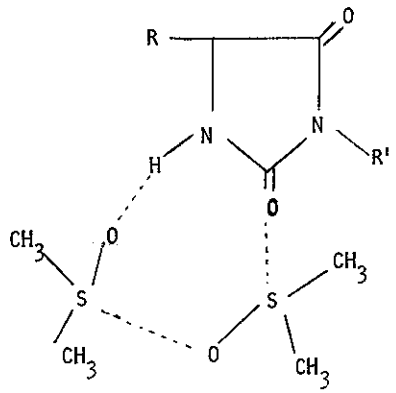
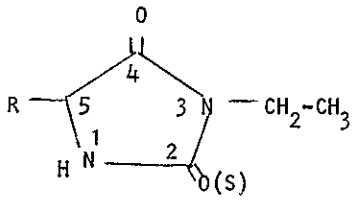


Table 4.

Proton Chemical Shift of the 3-Ethyl Group in Hydantoins and Thiohydantoins.



Substituent R	Solvent (D = DMSO-d_6, C = Chloroform)	Chemical Shift (ppm from TMS) CH_2	CH_3
H	D	3.42	1.09
	C	3.53	1.19
i - Pr	D	3.38	1.07
Ph*	D	3.78	1.13
$PhCH_2$	D	3.21	0.76
	C	3.43	1.02
(p)$ClC_6H_4CH_2$	D	3.21	0.79
	C	3.45	1.05
$PhCH_2$*	D	3.56	0.75
	C	3.78	1.05
$PhCH_2CH_2$	D	3.40	1.07
	C	3.57	1.20
Skatyl*	D	3.47	0.62
	C	3.78	1.03
H* (N-Me analog)	D		3.06
$PhCH_2$* (N-Me analog)	D		2.87
	C		3.13
$PhCH_2$ (N-Pr analog)	D		0.57
	C		0.83

NOTE: * denotes 2-thiohydantoin

compounds can be ascribed in part, to the attraction between this "dipolar pocket" and the π-electron-bearing substituent at C-5. Such attraction favors a "folded structure" in 5-benzyl compounds.

The conformation of 5-benzyl hydantoins can be represented by an appropriate combination of the three rotamers VIII-A, VIII-B, and VIII-C. Following the nomenclature used by Blout et al. (16), VIII-A can be termed as 'extended to O' conformation, VIII-B as 'extended to N' conformation, and VIII-C as the 'folded' conformation.

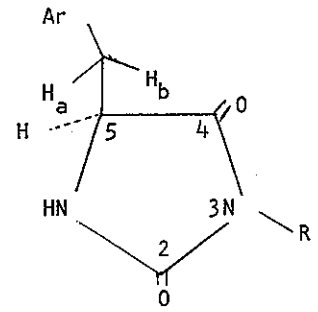
3.2 Proton Chemical Shifts of 3-Arlyhydantoins

In view of the well known restricted rotation of amide derivatives, it appeared to be of interest to study 3-tolylhydantoins. While this study was in progress Colebrook et al (17) reported the restricted rotation about the N-aryl single bonds. The structure of rotamers (IXα) and (IXβ) resulting from restricted rotation are shown in Figure 3.

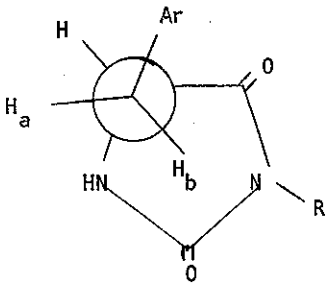
The chemical shift values reported in Table 5 reveal an obvious difference between the hydantoins derived from aliphatic amino acids and aromatic amino acids.

As expected from considerations of symmetry, the glycine derivative (IXa) shows a single peak for the methyl protons of the o-tolyl group. In the alanine and valine derivatives (IXb) and (IXf), two slightly separated methyl signals are observed. But in the hydantoins (IXd), and (IXg), and (IXh), there is clear evidence for the shielding of the methyl group due to the aromatic ring at C-5; the more downfield methyl signal appearing at a relatively fixed position can be ascribed to the rotamer (IX-α) while the higher field signal in varying positions is due to the rotamer (IX-β) (see Table 5).

Models show that in (IXc) the phenyl group cannot be as much over the hydantoin ring as in (IXd) or (IXg). Consequently, the two methyl peaks are closer together in (IXc) than in (IXd). In the case of (IXe) the $-CH_2-CH_2-$link allows multiple conformations some of which place the aromatic ring in close proximity to the amide groups of the hydantoin without being directly over the hydantoin ring. This accounts for the observation that only a single peak is seen for the methyl protons of the o-tolyl group in (IXe).

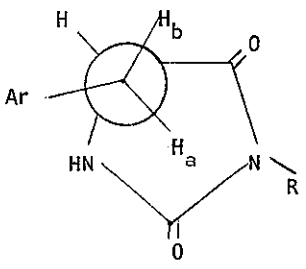


VIII



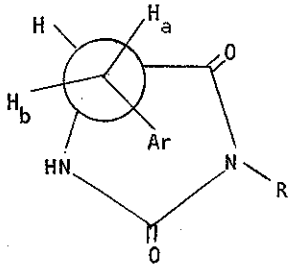
VIII-A

Extended to O



VIII-B

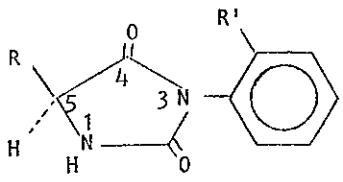
Extended to N

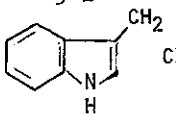


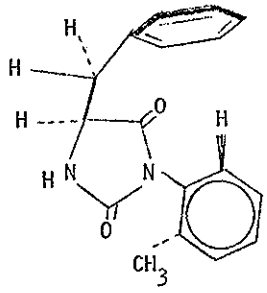
VIII-C

Folded

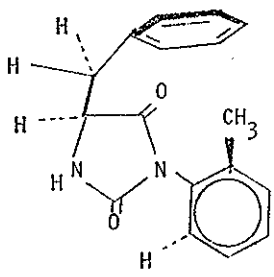
FIGURE - 3.



	R	R'		R	R'
IXa	H	CH_3	IXb	CH_3	CH_3
IXc	C_6H_5	CH_3	IXd	$C_6H_5CH_2$	CH_3
IXe	$C_6H_5CH_2$-CH_2	CH_3	IXf	$(CH_3)_2CH$	CH_3
IXg	(p)$ClC_6H_4CH_2$	CH_3	IXh		CH_3
IXi	$C_6H_5CH_2$	OCH_3			



IX-α



IX-β

Table 5

Proton and Carbon-13 NMR Spectra of 3-Arylhydantoins (IX) in DMSO-d_6

Compound*	R	(δ in ppm from TMS) (o)CH_{3d} [a]	(o)CH_{3u} [a]	(o)-H_d [b]	(o)-H_u [b]	Population ICH_{3u}	Ratio [d] ICH_{3d}
IXa	H		2.13 17.70	7.23			
IXb	CH_3	2.14 17.91	2.10 17.69	7.23	---	0.55	0.45
IXc	C_6H_5	2.21 17.34	2.07 17.07	7.27	---	0.63	0.37
IXd	$C_6H_5CH_2$	2.07 17.59	1.36 16.83	7.05	6.29	0.60	0.40
IXe	$C_6H_5CH_2$-CH_2	(2.20)[c]		(7.10)[c]		---	
IXf	$(CH_3)_2CH$	(2.12)[c] 18.02	 17.80	(7.13)[c]		---	
IXg	(p)Cl-C_6H_4-CH_2	2.10 17.70	1.43 16.83	7.10	6.37	0.62	0.38
IXh	$C_8H_6NCH_2$	2.08 17.26	1.11 15.97	7.00	6.03	0.62	0.38
IXi	$C_6H_5CH_2$	3.73 (55.89)	3.57	6.87	6.37	0.40	0.60

* In IXi, R_1 = OMe; in all other cases R_1 = Me

a) Subscripts d and u stand for the downfield and upfield methyl resonance, respectively.

b) Subscripts d and u stand for the downfield and upfield o-proton resonance, respectively.

c) Values in parenthesis indicate single peaks of the accidentally superimposed resonance.

d) The ratio was obtained by comparing the area under the upfield methyl (ICH_{3u}) peak with the area under the downfield methyl (ICH_{3d}) peak.

e) Underlined values indicate carbon-13 chemical shifts from TMS.

3.3 Rotamer Population from the Coupling Pattern of 5-Benzylhydantoins

The two benzylic protons (H_a,H_b) of 5-benzylhydantoins couple with 5-H on the heterocycle to produce a coupling pattern depending upon the solvent. In general, the ABX pattern is obtained in $CDCl_3$ solution while a DMSO solution gives an A_2X pattern (Table 6).

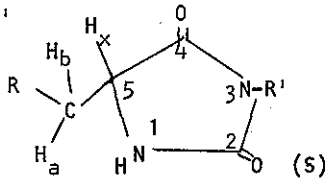
Assuming J_g and J_t to be 2.60 and 13.56 Hz, respectively, the approximate rotamer population was calculated from the average couplings observed. Since it was not possible to distinguish between the benzyl protons (H_a and H_b) without a labeling study, the population of the two types of extended conformers could not be distinguished. It was possible, however, to calculate the population density of the folded conformation vs. that of the two extended conformations (Table 7).

Most 3-aryl-5-benzyl hydantoin derivatives show an A_2X pattern in various solvents due to the vicinal coupling between the benzyl proton and the adjacent 5-proton in the hydantoin ring. The coupling constant and the three possible staggered rotamer populations are listed in Table 8. The standard coupling constants of J_g = 2.60 Hz and J_t = 13.56 Hz are chosen from the values of Pachler (5). The average coupling constant in DMSO-d_6 is slightly smaller than that in $CDCl_3$. Consequently, the population of the folded conformer is larger in DMSO-d_6 and the shielding effects are stronger than in $CDCl_3$.

The relative proportion of the rotamers (IX-α) and (IX-β) for various hydantoins can be determined by integration of the area under the two o-methyl peaks or the two o-proton peaks (see Table 5). It is interesting to note that the rotamer (IX-β) in which the o-methyl group is facing the substituent (aliphatic or aromatic) at C-5 is preferred over the rotamer (IX-α) in which the o-methyl group is far away from the C-5 substituent. But, the reverse situation prevails in the case of (IXi), the only difference here being the substitution of an O-Me group for Me.

Table 6

Vicinal coupling between benzyl protons and 5-H.



(S)

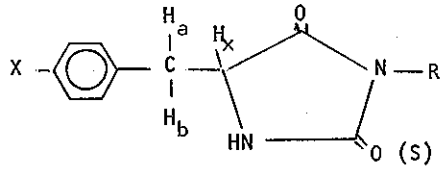
Substituent R	Substituent R'	Solvent (C=Chloroform) (D=DMSO-d_6)	J_{AX}	J_{BX} (Hz)	J_{AB}
C_6H_5	CH_3	C	8.37	3.91	-13.97
		D		(5.2)	
C_6H_5	CH_2CH_3	C	7.21	4.28	-13.80
		D		(4.8)	
(p)ClC_6H_4	CH_2CH_3	C	7.62	3.77	-14.14
		D		(4.8)	
C_6H_5	$CH_2CH_2CH_3$	C	7.38	3.86	-13.89
		D		(4.8)	
C_6H_5*	CH_3	C	8.20	4.10	-14.12
		D		(5.0)	
C_6H_5*	CH_2CH_3	C	8.11	4.26	-14.12
		D		(5.0)	
H	H	D		(7.0)	
		Acetone-d_6		(7.0)	
$C_6H_5CH_2$	CH_2CH_3	C	7.8	5.0	-
		D		(6.9)	

* 2-thiohydantoin derivatives

The values in parenthesis represent the coupling constant for A_2X pattern.

Table 7

Rotamer Population of 3-Alkyl-5-benzylhydantoins

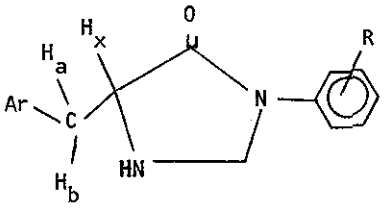


Substituents		Population Ratio**	
X	R	Extended Conformers	Folded Conformers
H	CH_3	0.63	0.37
H	CH_2CH_3	0.58	0.42
Cl	CH_2CH_3	0.57	0.43
H	$CH_2CH_2CH_3$	0.55	0.45
H*	CH_3	0.65	0.35
H*	CH_2CH_3	0.65	0.35

* 2-Thiohydantoin derivative.

** In $CDCl_3$ solution.

Table 8.



Substituents		Solvent[a]	Javg.	Population Ratio	
Ar	R		(Hz)[b]	Extended Rotamers	Folded Rotamer
C_6H_5	(o)CH_3	D	4.2	0.30	0.70
		C	4.8	0.40	0.60
		A	4.5	0.35	0.65
		N	4.5	0.35	0.65
C_6H_5	(m)CH_3	D	4.3	0.32	0.68
C_6H_5	(p)CH_3	D	4.7	0.38	0.62
		C	J_{ax}=6.5	0.44	0.56
		(ABX pattern)	J_{bx}=4.0		
(p)HOC_6H_4	(o)CH_3	D	4.2	0.30	0.70
(p)ClC_6H_4	(o)CH_3	D	4.5	0.35	0.65
C_6H_5	(o)OCH_3	D	4.6	0.37	0.63
		C	J_{ax}=6.5	0.49	0.51
		(ABX pattern)	J_{bx}=4.0		
C_6H_5	(p)OCH_3	D	4.5	0.35	0.65
C_8H_6N[c]	(o)CH_3	D	4.0	0.26	0.74
		A	4.8	0.40	0.60
C_8H_6N[c]	(p)CH_3	D	4.8	0.40	0.60
H	(o)CH_3	D	7.0	-	-
		C	6.9	-	-

a) D = DMSO-d_6, C = $CDCl_3$, A = acetone-d_6, N = nitrobenzene.

b) error is $\pm$ 0.05

c) Skatyl.

3.4 Temperature Dependence of the Proton NMR Spectra of Hydantoins

The chemical shift and the coupling constants of 3-alkyl and 3-aryl hydantoins change with temperature.

The most diamagnetically shielded α- proton in the 3-alkyl case is shifted significantly downfield when the temperature is raised. In contrast the chemical shifts of the deshielded 5-proton in hydantoin moves upfield as the temperature is increased. This is probably caused by the rotation of the 5-benzyl group as a function of temperature (see Table 9).

The average coupling constant (J_{avg}) of the benzyl proton with the adjacent 5-proton increases with temperature. Assuming that the standard coupling constant of J_g and J_t are temperature independent, the rotamer populations can be calculated (Table 9). It can be seen that the population of the folded rotamer decreases with rising temperature as is to be expected.

Temperature studies on 3-o-tolyl-5-benxyl hydantoin in DMSO solution show that the o-methyl and o-proton coalesce at 110°C. The o-tolyl group can rotate freely above this temperature.

Temperature studies (see Table 10) were made on several 3-tolylhydantoins. A representative spectrum is shown in Figure 4.

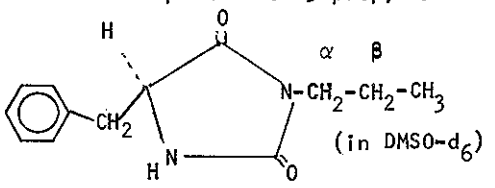
As temperature increases, the 5-benzyl (or 5-skatyl) also starts to rotate and the diamagnetically shielded peak moves rapidly downfield. The coupling constants of the benzyl protons with the adjacent 5-proton and the chemical shift of the o-methyl are changed when the temperature decreases in CD_3COCD_3 (see Table 11). The down field o-methyl peak is independent of the temperature. In contrast, the upfield methyl peak moves further upfield and at the same time the coupling constant (J_{avg}) decreases indicating the stability of the folded conformation at low temperature.

3.5 Solvent and Concentration Effect

The proton NMR spectrum of (IXd) was slightly different in the highly polar solvent DMSO-d_6 than in $CDCl_3$. The concentration of the solution too affected the spectrum. Some proton peaks of (IXd) shifted downfield while others moved upfield with increasing dilution of the chloroform solution. In particular, the upfield o-methyl and

Table 9a.

Temperature study of the PMR spectra for 3-propyl-5-benxylhydantoin.



Temperature °C	Chemical shifts (Hz from TMS on a 60MHz spectrometer) α-CH_2	β-CH_2	CH_3	-CH_2	CH	Ph	NH
40	188.3	72.0	34.5	179.5	260.5	432	483
60	189.0	73.5	36.0	179.0	258.0	-	-
80	189.2	75.0	37.0	179.0	255.8	-	-
100	189.5	76.0	38.0	178.5	256.6	-	-
120	190.7	77.5	39.0	179.0	255.6	-	457
150	190.5	79.0	40.3	178.5	253.0	429.5	-
	2.2	7.0	5.8	-1.0	-7.5	-2.5	-

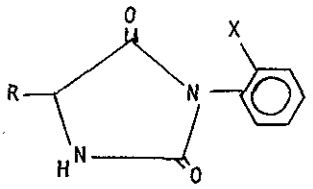
Table 9b.

Rotamer populations of 3-propyl-5-benxylhydantoin

Temperature °C	J_{avg} Hz	Population Ratio Extended Rotamers	Folded Rotamers
40	4.8	0.39	0.61
60	5.1	0.45	0.55
100	5.2	0.48	0.52
120	5.3	0.50	0.50
150	5.6	0.55	0.45

Table 10.

Coalescence Temperature in DMSO-d_6 Solution for 3-Arylhydantoins.



Substituent		Coalescence Temp.
R	X	°C
CH_3	CH_3	65
C_6H_5	CH_3	90
$C_6H_5NCH_2$	CH_3	110
$C_8H_6NCH_2$	CH_3	120
$C_6H_5CH_2$	OCH_3	80

Table 11

Temperature studies on 3-(o)-tolyl-5-benzylhydantoin in Acetone-d_6

Temperature	Chemical Shifts (Hz from TMS)		Coupling Constant
°C	(o)$CH_{3\ d}$	(o)$CH_{3\ u}$	J_{avg}
33	129.5	94.0	4.8
20	130.5	93.4	4.8
0	130.0	89.5	4.5
-20	130.7	87.6	4.5
-40	130.5	84.7	-
-60	131.0	82.0	4.1
-80	131.0	79.5	-

d) stands for the down field methyl

u) stands for the up field methyl

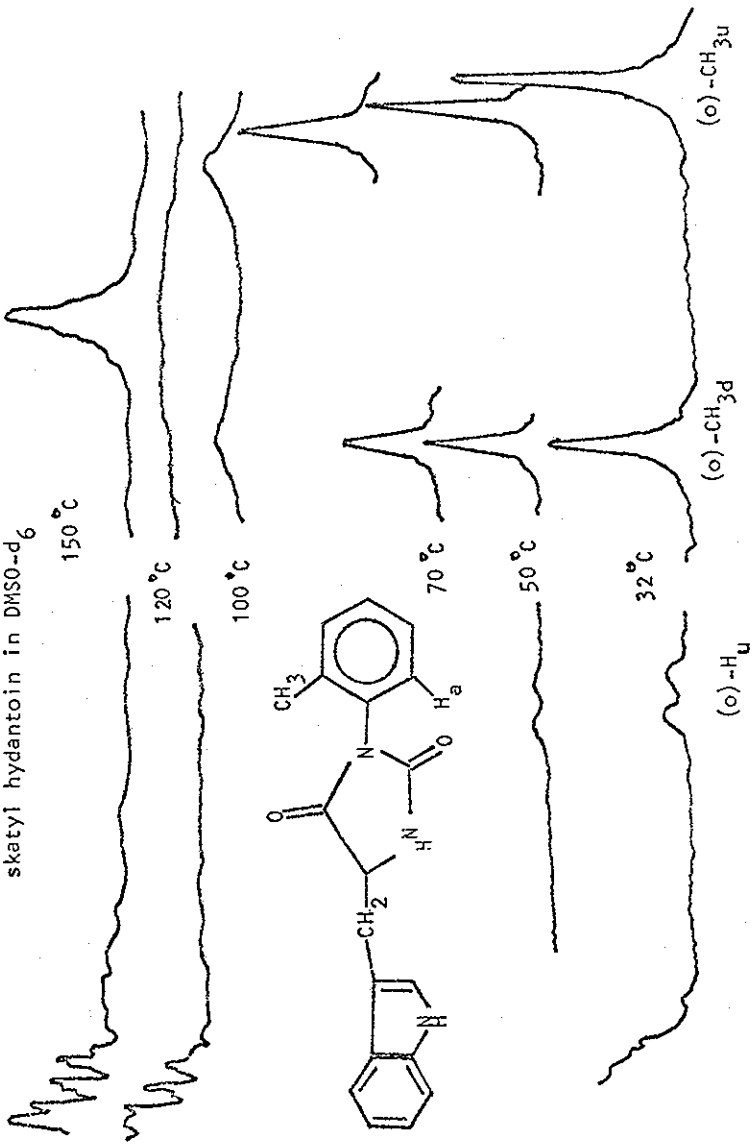

Temperature-dependent PMR spectra of 3-(o)-tolyl- 5-skatyl hydantoin in DMSO-d_6
150 °C
120 °C
100 °C
70 °C
50 °C
32 °C
(o)-H_u
(o)-CH_{3d}
(o)-CH_{3u}
CH_3
H_a
CH_2

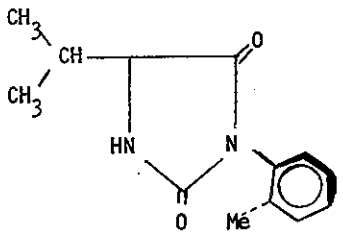
FIGURE 4.

o-proton peaks moved downfield rapidly on dilution but the downfield o-methyl signal remained unchanged.

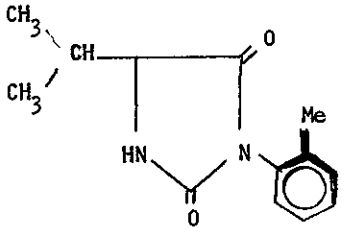
The dilution studies were made on (IXd) primarily to determine whether the non-bonded attraction was intermolecular or intramolecular. With increasing dilution, the higher field methyl peak moved to lower field but even in very dilute solutions there was a sizeable separation (22.5 Hz) between the two methyl signals. Hence, in chloroform solution there is evidence for intermolecular effects although the intramolecular part of the effect is of major consequence in influencing NMR resonances. In DMSO-d_6 solution, the concentration effect was minimal - indicating thereby that almost all of the attractive force was intramolecular. The concentration studies made on 3-ethyl-5-benzyl-2-thiohydantoin and 3-(m-tolyl)-5-benzylhydantoin in $CDCl_3$ solution (see Table 12) showed that the attractive force was essentially intramolecular. In DMSO-d_6 solution, the spectra of these hydantoins were unaffected upon dilution.

4. Carbon-13 NMR Studies

^{13}C NMR spectra are considerably more sensitive to the stereochemistry of the molecule. Thus, the ^{13}C NMR spectrum of the hydantoin (X) gave two distinguishable peaks for the aryl-Me group but the proton NMR spectrum at -80° and on a 270 MHz spectrometer failed to resolve the aryl-Me signal and distinguish between (Xα) and (Xβ).



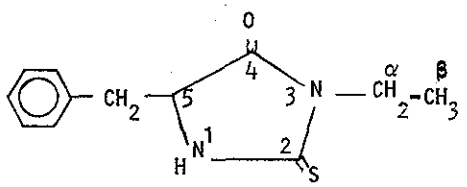
(Xα)



(Xβ)

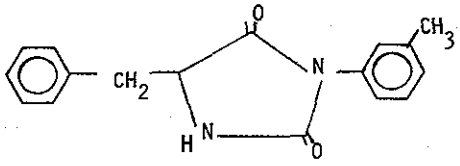
Table 12.

Concentration Studies (PMR Spectra in $CDCl_3$ on a 60 MHz spectrometer) 3-ethyl-5-benxyl-2-thiohydantoin.



Concentration	Chemical Shifts (Hz from TMS)				
	β-CH_3	α-CH_2	5-CH		NH
0.158M	64.5	226	257.5	434	450
0.079M	66.0	227	257.0	-	-
0.040M	66.5	228.5	258.0	435	-
0.020M	66.5	229.0	-	435	380

3-m-tolyl-5-benzylhydantoin



Concentration	Chemical Shifts (Hz from TMS)			
	(m)CH_3	5-CH	(o)H	NH
0.158M	140	257	415	400
0.079M	141.5	258	416	380
0.040M	141.3	260	418	360
0.020M	142.0	-	420	345
0.010M	142.5	-	420	-

For hydantoins asymmetric at C-5, the ^{13}C NMR spectrum displayed two peaks for the aryl-methyl group. The carbonyl resonance too were resolved in many cases but most other peaks were not resolved.

In recent publications, we have shown titanium tetrachloride (18) to be an effective shift reagent for studying the ^{13}C NMR spectra of carbonyl compounds. When we added this reagent to N-tolylhydantoin solutions, the C-2 peak was shifted downfield but the C-4 carbonyl resonance was unchanged. These observations indicate that titanium tetrachloride is coordinated effectively only with the HN-CO group but there is little interaction with the other carbonyl in the molecule.

The difference in the chemical shift between the two resonances of the methyl of the arylmethyl group increases considerably when the substituent at C-5 is a benzyl group indicating substantial contribution from the folded conformation of the hydantoin.

Various NMR methods have been applied to cyclic dipeptides (see Section 7) to elucidate their structural aspects. Deslauriers and coworkers (30) have reported on ^{13}C NMR studies, in particular, the measurement of ^{13}C spin-lattice relaxation times (T_1). The latter parameters are sensitive to the presence of solvent molecules which are strongly hydrogen bonded to the cyclic dipeptide, such as cyclo (L-Tyr-Gly). The effects of the aromatic "ring currents" on ^{13}C chemical shifts in some cases were overshadowed by the small changes in geometry of the diketopiperazine ring.

Blout and coworkers (16) have applied lanthanide assisted ^{13}C and ^{1}H NMR analysis to the determination of the preferred conformation of proline-containing cyclic dipeptides. The chemical shift and coupling constant data indicated that aliphatic side-chains adopt rotamers favored by steric considerations, whereas aromatic side chains assume folded conformations due to additional stabilizing interaction, (also see Section 7).

TABLE 13

Crystallographic Data

Compound	1a	XIa	XIIb
Formula	$C_{23}H_{22}N_2O$	$C_{17}H_{15}N_2O_2Cl$	$C_{12}H_{14}O_2N_2S$
Mol. Wt.	342	314.77	250.31
a	12.976(4)	12.985(5)	5.488(3)
b	9.632(4)	7.633(3)	15.705(4)
c	16.090(5)	17.035(8)	14.865(4)
β	106.31(1)	98.90(3)	101.45(5)
Space Group	$P2_1/n$	$P2_1/c$	$P2_1/c$
Calc. Density		1.35 g/cm^3	1.31 g/cm^3
Exp. Density		1.35 g/cm^3	1.31 g/cm^3

5. X-ray Crystallographic Studies

Over 35 hydantoins (XI) have provided NMR evidence for a preferred folded conformation as shown by abnormal shielding in R_2 groups. The only requirement is a benzyl group in the 5 position and some convenient marker in R_2.

5 phenyl groups do not show extra shielding in R_2 because it is geometrically impossible for the phenyl ring to lie over the R_2. A phenyl ethyl group at 5 does not show the effect because many of the multiple conformations possible place the R_2 group outside the shielding cone.

In order to investigate these effects we chose to investigate two hydantoins in the solid phase, XIa R_1 = Cl, X = O, R_2 = O-tolyl) and XIb (R_1 = OH, X=S, R_2 = Et). Although several other derivatives were potentially interesting, most compounds of type XI yielded twinned or otherwise unsatisfactory crystals. Recently we have also determined the structure of the compounds which originally excited our interest in aromatic dipole interactions, IA (n=1).

Table 13 summarized the basic crystallographic data for the three compounds whose structures were solved and refined by standard methods. It was somewhat disconcerting at first to find that only XIb showed the folded conformation expected from the NMR studies (see Figure 5).

In XIa the distance Between the o-methyl group in the 3-N-tolyl substituent and the plane of the p-chloro substituted benzyl ring at position 5 is 3.56Å. The Bovey Z and P coordinates are 2.56Å and 0.36Å respectively which places the methyl in the shielding cone of the phenyl ring. Calculations of the Z and P coordinates from solution NMR data yield Z and P values of 2.50 and 1.00. The results from solid and solution are thus in reasonable agreement for XIb.

The extended conformation of XIb (Figure 6) is probably caused by the hydrogen bond formed between the p-hydroxy group on the 5-benzyl group and the 4 carbonyl oxygen in the hydantoin ring of an adjacent molecule. The packing diagram in Figure 7 shows this clearly. Apparently the hydrogen bond energy was more important than the stabilization due to the aromatic amide conformation. However, it is important to note that the 5-benzyl group is oriented directly over the hydrogen bond N(2)-H... S' between N(2) in the molecule in which the benzyl group is located and S' in an adjacent molecule (Figure 8). This preference of the phenyl ring for the polar regions of the crystal structure has important implications for the maintenance of a preferred conformation in aromatic ring containing peptides and proteins.

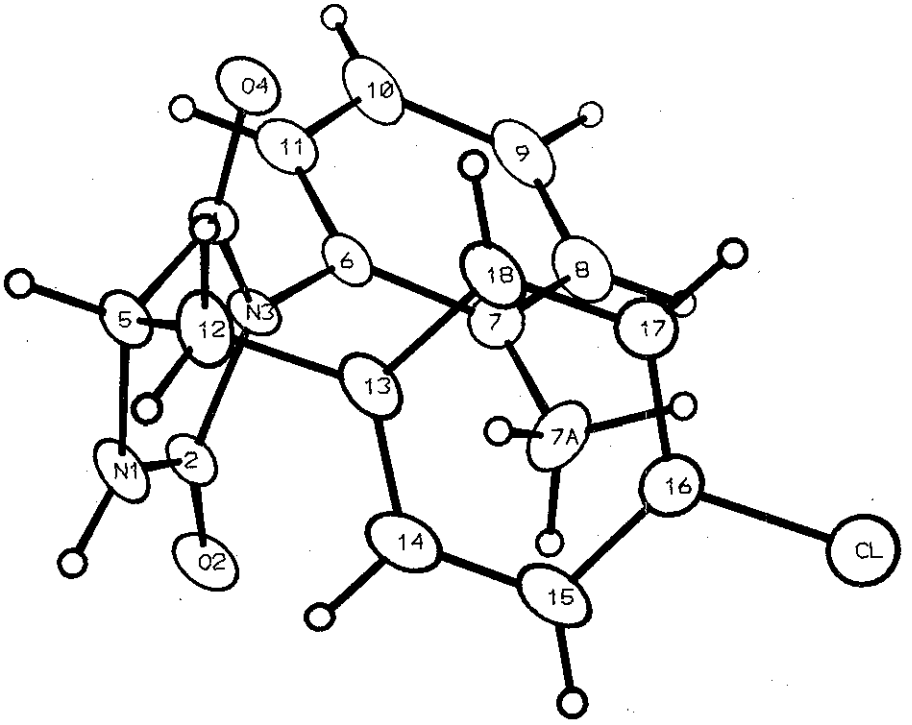
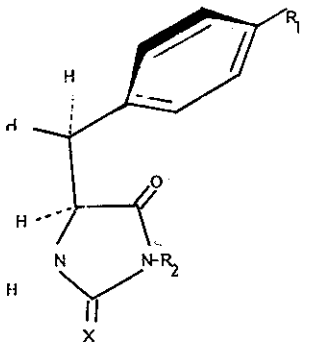
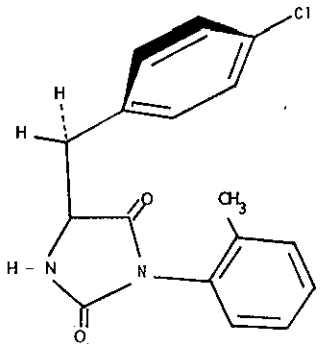


FIGURE 5 Folded conformation of hydantoin XIa.

The structure of 1a (n=1) was similarly planar (Figure 9) and not folded. Here there is no hydroxyl group which might offer an explanation of a stronger packing force. However, again it should be noted that there is an intermolecular attraction of the phenyl groups for the polar regions of the crystal (Figure 10).



XI.



XI (a).

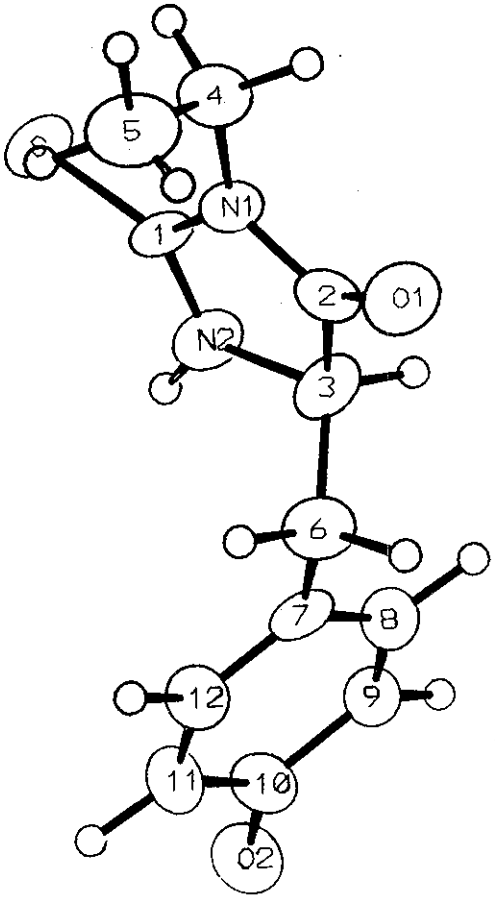


FIGURE 6 Extended Conformation of Compound XIb

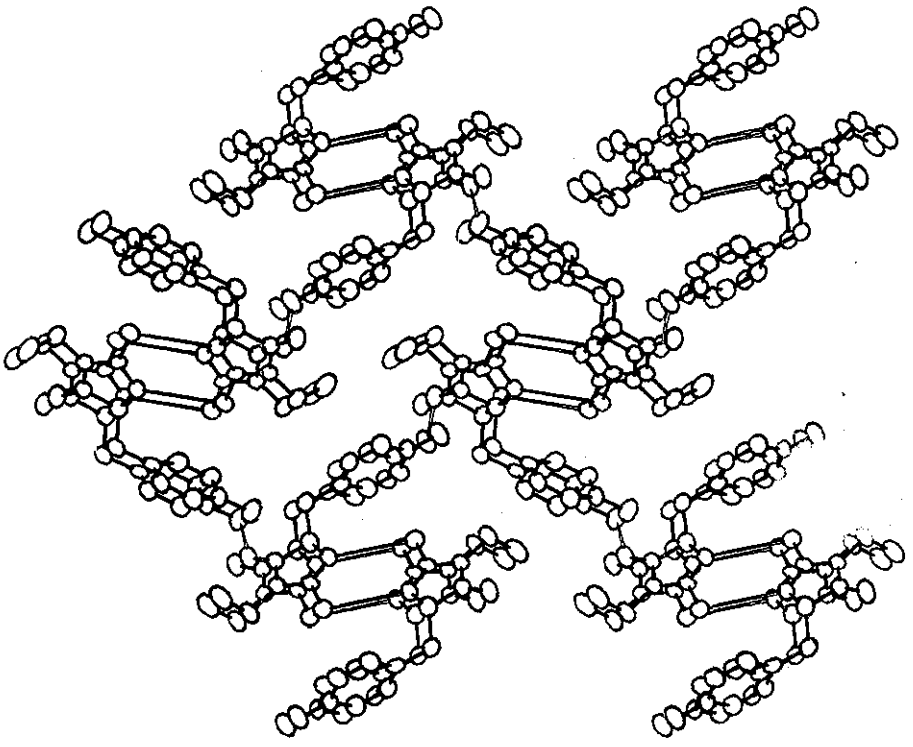


FIGURE 7 Packing diagram of hydantoin XIb showing the interaction of the phenyl ring with the hydrogen bonded region.

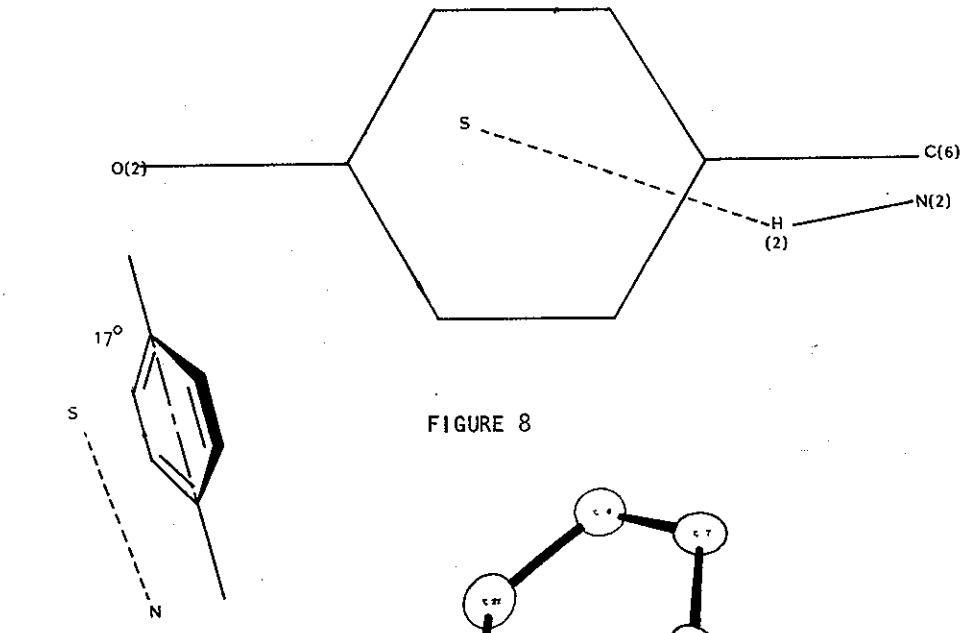


FIGURE 8

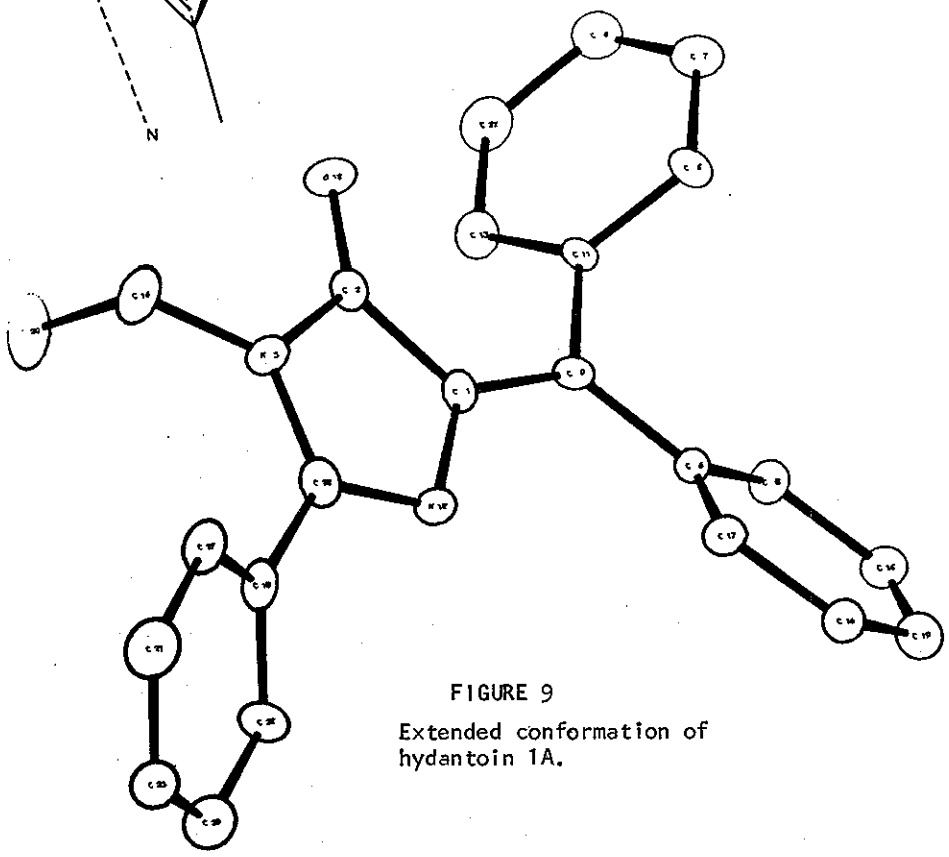


FIGURE 9

Extended conformation of hydantoin 1A.

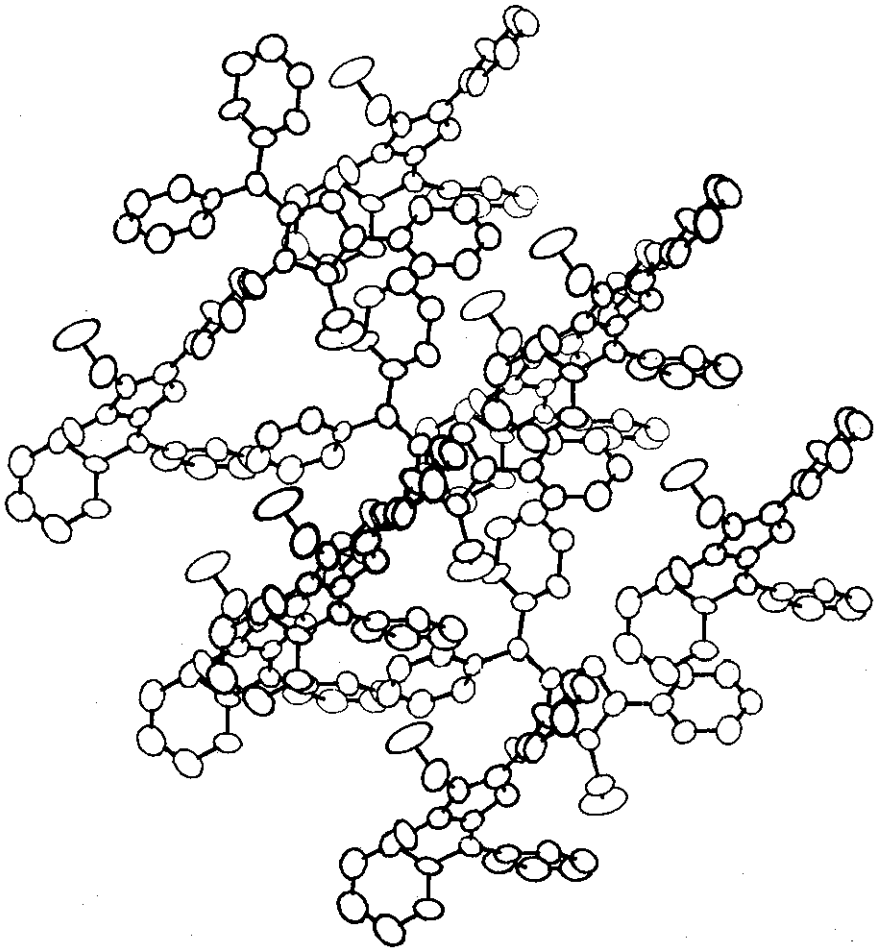


FIGURE 10 Packing diagram of hydantoin IA.

6. Conformation of 5-Benzylhydantoins in Solution

The NMR studies reported here give clear indication for a folded conformation for 5-benzylhydantoins in solution. The findings from X-ray diffraction studies too provide support for this conformation. To explain the concentration effect observed in $CDCl_3$ solution but not in DMSO-d_6 solution, it has been suggested in an earlier section that hydrogen bonding involving the -NH-CO- group in the hydantoin ring creates a 'dipolar pocket'. Non-bonded attraction between the π -electrons in the 5-benzyl group and this 'dipolar pocket' becomes important in concentrated solution. In dilute solutions this attraction disappears but the aromatic ring of the benzyl group is still folded over the hydantoin ring because of the intramolecular non-bonded interaction between the π -electrons and the dipole of the ring system.

The observed relative proportion of rotamers (IX-α) and (IX-β) are not easily accounted for. It is possible that the (IX-β) rotamer is favored over the (IX-α) rotamer because of hydrophobic interaction between the o-methyl group and the benzyl group. In the case of (IXi), it then becomes necessary to assume that the proximity of the hydrophilic oxygen of the OCH_3 group changes the rotamer population in favor of the (IX-α) rotamer.

It has been noted earlier that there is no chemical shift difference in the ^1H NMR spectrum of (IXd) in DMSO-d_6 solution upon dilution. The amide proton (NH) peak appears at about 7.90 ppm as a broad peak, thereby indicating considerable hydrogen bonding. It is suggested that the -CO-NH- group of the hydantoin ring is hydrogen bonded to the solvent-thus excluding the intramolecular hydrogen bonding postulated for $CDCl_3$ solution.

Temperature studies of the NMR spectra of hydantoins indicate the rotation of the 3-o-tolyl and the 5-benzyl groups and also supports the folded structure.

7. Conformation of Diketopiperazines

The conformation of cyclic dipeptides or diketopiperazines have received considerable attention since 1967. In that year, Kopple and Mar (19) and Blaha and Samek (20) published proton NMR studies on diketopiperazines derived from an aromatic and an aliphatic amino acid, or two aliphatic amino acids. Kopple and Mar concluded that the aromatic ring lies folded over the diketopiperazine

ring and thereby causes upfield shift of the α-protons of the other amino acid component of the diketopiperazine. Subsequent papers by Kopple and coworkers (21) and also others (22) have firmly established that the folded conformer is favored over the extended conformers. The attraction between rings that stabilizes this folded conformation is not significantly dependent on solvents. Ziauddin and Kopple (23) studied the proton NMR spectra of 3-(p-methoxybenzyl)-, 3-(p-nitrobenzyl)-, and 3-benzylpiperazine-2,5-diones relative to corresponding protons of piperazine-2,5-diones lacking an aromatic ring. The solvents used were dimethyl sulfoxide, trifluoroacetic acid, hexafluoroacetone sesquidenterate, and acetic acid. The temperature dependences of these differences were used to estimate the enthalpy of stabilization of the folded conformation and the extent to which the cis 6-H is shielded in that conformation. From these measurements it was concluded by Ziauddin and Kopple that neither an electron withdrawing substituent ($-NO_2$) nor an electron donating (-OMe) substituent made any appreciable contribution to the stability of the folded form. An explanation of the observations in terms of the role of solvents was not obvious.

Gawne et al. (24) have studied the proton NMR spectra of several diketopiperazines as well as their N-methyl derivatives. In the 100 MHz NMR spectrum of 2-benzyldiketopiperazine in trifluoroacetic acid solution, the two α-protons of the glycyl residue appear as a widely separated (6.96 and 6.06 ppm) AB quartet. In the NMR spectrum of diketopiperazine from leucylglycine which was used as a reference compound, both glycyl α-protons appear together as a singlet at 5.64 ppm. The shielding observed is larger than that calculated based on the theoretical shielding data of Johnson and Bovey (25) even after assuming that the diketopiperazine was *entirely* in the folded form. For these calculations the heterocycle ring was considered to be planar as found by X-ray crystallography for cyclo-glycylclycine. However, if the diketopiperazine ring was assumed to have a boat form with the benzyl group occupying a "flagpole" rather than a "bowsprit" orientation, the experimentally determined large shielding could be accounted for. In support of the non-planar heterocyclic ring, it was observed that the more downfield doublet assigned to the trans proton of the glycyl residue was broader ($W_{\frac{1}{2}}h$ = 6Hz) than the doublet ($W_{\frac{1}{2}}h$ = 3Hz) for the cis proton. This difference in width of the signals disappeared when deuterated trifluoroacetic acid was used as the solvent ensuring N-D formation.

Obviously, the trans and cis protons are unequally coupled with the adjacent N-H - a situation indicative of a non-planar heterocyclic ring. The vicinal H-H couplings for the benzylic protons with 2-H were nearly equal (5-Hz) indicating thereby that the phenyl ring faced the diketopiperazine ring which itself was buckled to place the benzyl ring in the flagpole orientation.

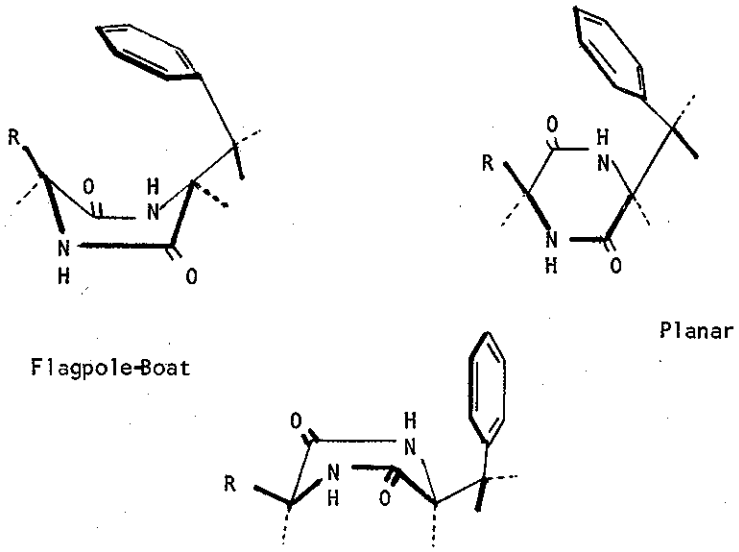
N-methylated diketopiperazines are soluble in chloroform as well as trifluoroacetic acid and D_2O. Shielding effects observed for them were somewhat larger in the polar solvents. From temperature studies, it was estimated that the enthalpy difference between the folded and unfolded conformations in chloroform solution was of the order of 3 kcal/mole. Similar small values had been reported by Kopple and Mar for cyclo-(L-tyrosylglycine).

X-ray crystallography of a number of dipeptides have been reported. The simplest diketopiperazine is planar but the replacement of one or both glycines changes this shape. Of course, in the crystals the molecular shape has to accommodate close packing, hydrogen bonding, intermolecular stacking interactions, etc.

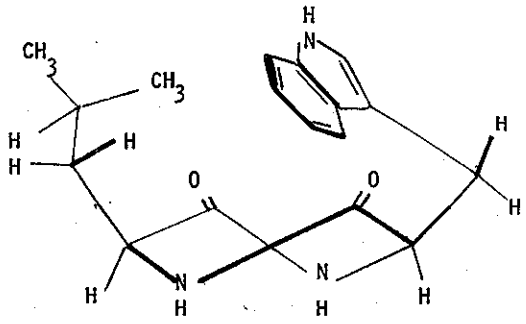
In case of cyclo-(D-Ala-L-Ala) the diketopiperazine ring is essentially planar but in cyclo-(L-Ala-L-Ala) the heterocyclic ring assumes a boat conformation of the bowsprit type. In cyclo-(L-Pro--(L-Pro-L-Leu) too is in the same form with a large deviation from planarity.

Lin and Webb (26) have generalized the relationship between amino acid sequence and the diketopiperazine ring conformation. The two most important factors are the maximization of the interaction of the aromatic ring with the heterocyclic ring and the avoidance of side-chain steric interference. Thus, cyclo-(Gly-X) where X is aromatic, is expected to be flagpole boat. In cyclo-(L-Y-L-X), the introduction of the Y residue prevents the flagpole form; unless Y is too bulky, a nearly planar diketopiperazine ring allows interaction between X and the heterocycle without interference between X and Y.

Caillet, Pullman and Maigret (27) have carried out molecular orbital calculations and concluded that in cyclo-(Gly-Phe), the folded conformation is indeed the most stable. In case of cyclo-(Gly-Val) of valine over the diketopiperazine ring although this preference is less pronounced than in cyclo-(Gly-Phe).



Conformations of 2-benzyldiketopiperizines



Possible conformation of cyclo(L-leucyl-L-tryptophyl)

It is generally accepted that the attraction between the aromatic ring in diketopiperazines is between the polarizable π-electrons and the dipole in the heterocyclic ring. The same situation obtains in hydantoins and in benzyl substituted derivatives of 4,6-dioxohexahydropyrimidines - compounds analogous to diketopiperazines in terms of the presence of polar NH-CO links. Even in the absence of a heterocyclic structure as in these compounds, the dipoles in the neighborhood of the aromatic ring of an amino acid may affect the conformation of a peptide. Deber and Joshua (28) have studied the 100 MHz proton NMR spectra in D_2O solution of a series of linear dipeptides of the types L-Phe-L- and -D-X, and L-Phe-L- and -D-Y, where X comprised a group of amino acid residues with polar side-chain (X=glutamine, glutamic acid, arginine, etc.) and Y comprised amino acid residues with purely aliphatic side-chains (Y=α-aminobutyric acid and norvaline). They observed that regardless of the side-chain length, peaks due to the Y-methylene protons in the X and Y side-chains of the L-Phe-D-X and L-Phe-D-Y series exhibited upfield shifts greater than any other protons in the side-chains, when compared to the corresponding side-chain resonances of the non-aromatic dipeptides L-Ala-L-X and L-Ala-L-Y. These shielding effects were considerably larger for the L-Phe-D-X series than for the L-Phe-D-Y series. An intramolecular complex - formed by the interaction of aromatic π-electrons with the positive end of the dipole in the polar side-chains. The attractive force was estimated to be small - an increase in temperature to 70-80°C was sufficient to overcome the enhanced shielding due to the complex form.

In hydantoins and diketopiperazines, these attractive forces are far stronger.

8. Biological Implications

Non-bonded attraction observed in aromatic amino acid derivatives is likely to be a factor in determining the tertiary structure of proteins including enzymes. A change in structure could significantly modify the physiological characteristics of biopolymers. Such modifications are likely to be more pronounced in small peptides rather than large oligopeptides and proteins.

Kopple and coworkers (2) have observed that in the reaction of o-nitrophenyl acetate with cyclo-(L-His-L-Tyr) fast acetyl transfer occurs from histidylmidazole to tyrosyl hydroxyl. This facile intramolecular acyl transfer is understandable in the light of the proximity of the groups involved in the folded conformation. It is

an interesting possibility that non-bonded attraction of histidine, phenylalanine, tryptophane and other aromatic amino acids with polar groups in their environment may play a role in modifying the rate of reactions at some active centers of enzymes (32).

Shiba and Nunami (29) have studied a group of bitter tasting diketopiperazines. The most bitter of them all was cyclo-(L-Leucyl-L-tryptophyl) which had been isolated from casein hydrolyzate. In CD_3OD or DMSO solution, the two α-methylene protons of leucine appear at abnormally high field: 0.70 and - 0.10 indicating that the side-chain of leucine is located very close to the indole ring. Coupling constants of 2.4 and 2.3 Hz due to NH protons of leucine and tryptophane respectively were interpreted as indicating a twist boat form for the diketopiperazine ring. In the conformation proposed for this compound, the two hydrophobic side-chains are in close proximity on one side of the molecule while the hydrogen bonding groups are located on the other side. Shiba and Nunami suggest that the bitter taste may be related to the stereochemical features of the molecule in solution.

9. Conclusions

Several interesting points have emerged from the studies on aromatic amino acid derivatives such as diketopiperazines and hydantoins. X-ray crystallographic studies have clearly indicated that non-bonded attraction can stabilize folded conformation for these heterocycles eventhough considerable steric hindrance may have to be overcome. Comparison of proton NMR data with X-ray crystallographic data have revealed that the favored conformation in the crystalline structure may be *different* from that in solution. In a crystal, the effect of intramolecular non-bonded interaction may be counter-balanced by other factors such as lattice forces, hydrogen bonding and intermolecular interaction. In a solution, the nature of the solvent may or may not have any significant effect on the rotamer population; the relative importance of the intra- versus inter-molecular interaction will of course depend on the concentration. These studies show that it is essential to take into consideration the non-bonded attraction between aromatic systems and suitably attraction between aromatic systems and suitably disposed dipoles such as amido groups in deducing the conformation of peptides in solution and even substrate-enzyme binding

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Received, 29th August, 1977