PROTON MAGNETIC RESONANCE STUDY OF THE CONFORMATION OF THE PYRROLIDINE RING IN SOME PROLINE-THIOHYDANTOINS

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Received February 3rd, 1988
Accepted June 6th, 1988

Dedicated to the memory of Dr Karel Bláha.

The conformational behaviour of a series of N-substituted thiohydantoins of proline was studied by proton NMR. The pseudorotational parameters of the proline moiety were calculated from the ten vicinal proton–proton coupling constants assuming a two state equilibrium (N ⇋ S). The effect of the different substituents (methyl, phenyl, *para*-nitrophenyl) at the thiohydantoin nitrogen on the conformation of the pyrrolidine rings is discussed. The spectra of proline-N-methylthiohydantoin were recorded at different temperatures in octadeuterotoluene solution. The conformational analysis of these spectra showed that the ratio of the populations of the N and S forms changes considerably with temperature, while the conformational identity of both the N and S forms remains strictly preserved. These facts provide an additional experimental justification of the two-state assumption, at least so for the present model compounds.

Since the development of the generalized Karplus equation by Haasnot et al.[1,2], a more reliable evaluation is feasible of torsion angles from experimental vicinal interproton coupling constants. When this equation is combined with the concept of pseudorotation[3–5] and the existence of two stable conformations is assumed in addition, a most valuable comprehension of the conformation of five-membred ring systems emerges. In this approach the pseudorotational phase angle (P) and the maximal pucker (τ) for the individual conformers N (North) and S (South) and their relative abundance (x_N) can be calculated by a least-squares iteration procedure. The quality of the fit is expressed as the rms value, based on the difference between experimental and calculated coupling constants.

The fact that lower rms values are obtained with the bistable state model (five parameters: P_N , τ_N, P_S, t_S and x_n) as compared to the single state

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approximation (only 2 parameters: P, τ) is trivial and does not guarantee the physical significance of the computed result. Confidence in the latter must be found elsewhere.

In this perspective we recorded high resolution proton NMR spectra of proline-N-methylthiohydantion at 360 MHz in octadeuterotoluene at temperatures of $-30°C$, $-5°C$, $20°C$, and $60°C$. The ten vicinal coupling constants of the pyrrolidine ring, refined by computer simulations of the 7-spin system, were subjected to conformational analysis as described (vide supra). It was found that a shift in the N $\rightleftharpoons$ S equilibrium is at the origin of the changes of the observed coupling constants with temperature. The conformational identity of the individual conformations however was not at all affected.

Similarly some N-substituted-thiohydantoins of proline (with the substituent hydrogen, methyl, phenyl, *p*-nitrophenyl) were investigated at room temperature. The difference in N-substitution of the thiohydantoin caused a small impact on the pyrrolidine ring conformation, i.e. a slight shift in the population of the N and S forms. This conformational drift can be explained by the typical canonical forms of the thioamide moiety of the thiohydantoin ring.

EXPERIMENTAL

Synthesis of the Substituted Proline Thiohydantoin

Proline (1·6 mmol) was dissolved in a mixture of 0·5 M aqueous sodium carbonate (25 ml) and dioxane (25 ml). Appropriate alkyl or aryl isocyanate (3·2 mmol) was added, the mixture was stirred for 3 h at room temperature and then refluxed for 1 h. After cooling to room temperature the mixture was acidified with concentrated hydrochloric acid to pH 1·2, refluxed for 2 h and then evaporated to dryness on a rotatory evaporator in vacuo (2·7 kPa). The residue was extracted with chloroform (50 ml), the chloroform solution was dried with anhydrous magnesium sulfate and evaporated to dryness; yield 60–80%. The unsubstituted hydantoin was synthesized according to the procedure described by Elmore et al.[6].

Melting points (Kofler hot plate, uncorrected): *Ia*: 50·0–50·5°C (ref.[7]: 51°C); *Ib*: 171·5–172·5°C (ref.[8]: 171–173°C); *Ic*: 179·0–179·5°C (ref.[9]: 179–180°C); *Id* is a new compound: m.p. 163·5–164°C; TLC on precoated (0·25 mm) silica plates, Merck DC-Fertigplatten Kieselgel $60F_{254}$, $R_F = 0·60$ (benzene–ethyl acetate, 1 : 1). IR (1% in KBr, cm^{-1}): 3 050, 3 038, 3 029, 3 020 (arom. ν_{C-H}), 2 989, 2 978, 2 947 (aliph. ν_{C-H}), 1 760 ($\nu_{C=O}$), 1 606, 1 592 (arom. $\nu_{C=C}$), 1 518, 1 497 (thioureide band), 1 420, 1 350, 1 268, 1 235, 1 168, 1 100, 1 042, 1 010, 960, 846, 740. $[\alpha]_{589}$ −18·35° (*c* 0·1; $CHCl_3$). For $C_{12}H_{11}N_3O_3S$ (277·3) calculated: 51·98% C, 4·00% H, 15·15% N, 11·56% S; found: 52·0% C, 4·2% H, 15·1% N, 11·4% S. For NMR spectra see Tables I and II.

NMR Measurements

The ^1H NMR spectra were recorded on a Bruker WH360 spectrometer. The digital resolution was 0·09 Hz/point for proline-N-methylthiohydantoin. Spectra were recorded of an octadeuterotoluene

solution at temperatures of −30°C, −5°C, 20°C, and 60°C. Unfortunately, the resolution of spectra recorded outside this temperature range, was not sufficient to guarantee accurate measurements of the coupling constants. The spectra of all other proline thiohydantoins were recorded in hexadeuterobenzene at a probe temperature of 22°C.

The initially obtained NMR parameters were refined using the standard BRUKER simulation program "PANIC". The resulting NMR data (Tables I and II) are based on the assignment of well-resolved and sharp lines to about 70% of the theoretical transitions with a minimum intensity of 0·001. The rms deviation between the experimental and the calculated transitions was in the range 0·16 ± 0·02 Hz.

The vicinal coupling constants obtained by simulation were used as input values for the computer program CONFIT[10]. This program combines the generalized Karplus equation[1], the concept of pseudorotation[3-5] and the two-state approximation in a least squares procedure[11]. The

TABLE I

Proton chemical shifts[a] (δ, in ppm downfield relative to internal TMS) of the N-methylthiohydantoin of proline (*Ia*) in toluene at different temperatures and of the N-substituted thiohydantoins of proline in benzene at room temperature

Comp.	*T*, °C	α	β^c	β^t	γ^c	γ^t	δ^c	δ^t
Ia	−30	3·025	0·427	1·167	1·077	0·932	3·439	2·830
	−5	3·115	0·600	1·249	1·168	1·024	3·499	2·853
	20	3·189	0·731	1·319	1·243	1·100	3·556	2·878
	60	3·309	0·919	1·434	1·362	1·223	3·651	2·927
Ib	22	3·263	0·933	1·427	1·335	1·130	3·360	2·904
Ic	22	3·366	1·019	1·447	1·342	1·171	3·656	2·966
Id	22	3·325	0·966	1·435	1·349	1·186	3·606	2·943

[a] Protons are indicated in the column headings by Greek letters (see structure *I*), with the superscript ^{c} (or ^{t}) to designate a proton that is *cis* (or *trans*), i.e. on the same (or opposite) side of the pyrrolidine ring as compared to the α-carboxamide group.

TABLE II

Proton coupling constants (in Hz) of the N-methylthiohydantoin of proline (*Ia*) at different temperatures and of the N-substituted thiohydantoins of proline at room temperature

Comp.	*T*, °C	$\alpha\beta^t$	$\alpha\beta^c$	$\beta^t\gamma^t$	$\beta^t\gamma^c$	$\beta^c\gamma^c$	$\beta^c\gamma^t$	$\gamma^c\delta^c$	$\gamma^c\delta^t$	$\gamma^t\delta^c$	$\gamma^t\delta^t$	$\beta^c\beta^t$	$\gamma^c\gamma^t$	$\delta^c\delta^t$
Ia	−30	6·86	10·64	1·86	6·86	7·74	11·73	7·88	2·57	9·25	9·12	−12·28	−12·84	−11·48
	−5	7·04	10·52	2·07	6·98	7·85	11·28	7·92	3·00	8·98	9·04	−12·32	−12·90	−11·48
	20	7·01	10·33	2·40	6·96	7·86	10·96	7·90	3·31	8·78	8·86	−12·35	−12·65	−11·48
	60	7·37	10·04	2·75	7·16	7·99	10·33	7·92	3·71	8·42	8·71	−12·30	−12·98	−11·66
Ib	22	6·96	10·46	2·42	7·02	7·87	10·97	7·94	3·35	9·00	8·82	−12·25	−12·84	−11·50
Ic	22	6·88	10·49	2·16	6·89	7·91	11·24	7·91	2·99	8·91	9·01	−12·26	−12·89	−11·83
Id	22	6·91	10·51	1·97	7·00	7·92	11·31	7·97	3·16	8·96	9·46	−12·18	−12·97	−11·83

conformational parameters (Tables III and IV) are obtained as output values. For all molecules investigated in this study calculations based on the bistable state model always resulted in a much lower rms value than the fixed single state model.

TABLE III

Conformational parameters (maximal pucker τ and pseudorotational phase angle P, for the North (N) and South (S) forms, and their relative abundance x) of the N-methylthiohydantoin of proline (*Ia*) at different temperatures and of the N-substituted thiohydantoins of proline at room temperature. Values in brackets are standard deviations

Comp.	T, °C	τ_N	P_N	τ_S	P_S	x_N	rms[a]
Ia	−30	41·9	−9·0	40·9	244·3	87·7	0·47
		(2·7)	(6·0)	(26·0)	(30·4)	(4·4)	
	− 5	41·9	−8·7	40·9	245·1	84·1	0·47
		(2·9)	(6·4)	(20·1)	(29·1)	(5·9)	
	20	43·1	−13·2	42·3	242·6	79·1	0·43
		(3·0)	(5·8)	(15·6)	(21·1)	(4·3)	
	60	41·6	−8·3	41·7	238·5	77·7	0·44
		(2·8)	(5·9)	(13·4)	(18·6)	(4·7)	
Ib	22	42·7	−8·4	41·4	245·2	81·1	0·49
		(3·1)	(6·7)	(17·7)	(20·1)	(4·5)	
Ic	22	42·3	−9·6	41·1	243·9	83·8	0·46
		(2·8)	(7·1)	(19·4)	(22·3)	(4·3)	
Id	22	41·5	−11·6	41·3	241·2	85·4	0·55
		(6·1)	(9·2)	(23·2)	(25·7)	(3·9)	

[a] rms = root mean square deviation between experimental and calculated coupling constants in Hz.

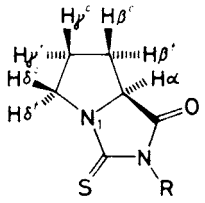
TABLE IV

Endocyclic torsion angles χ of the N and S forms of the pyrrolidine ring in the N-methylthiohydantoin of proline (*Ia*) at different temperatures and of the N-substituted thiohydantoins of proline at room temperature

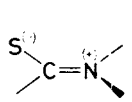
Comp.	T, °C	χ_1^N	χ_2^N	χ_3^N	χ_4^N	χ_5^N	χ_1^S	χ_2^S	χ_3^S	χ_4^S	χ_5^S
Ia	−30	−37	41	−30	7	19	−7	−18	36	−42	30
	− 5	−37	41	−30	7	19	−8	−17	36	−41	30
	20	−40	42	−28	4	22	−6	−19	38	−42	30
	60	−37	41	−30	7	18	−3	−22	39	−41	27
Ib	22	−38	42	−30	7	19	−8	−17	36	−41	30
Ic	22	−38	42	−30	6	20	−7	−18	36	−41	30
Id	22	−38	41	−28	5	20	−5	−20	37	−41	28

RESULTS AND DISCUSSION

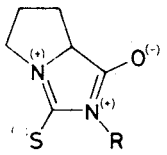
The proton chemical shifts and the $^1H-^1H$ coupling constants (as refined through simulation), of proline-N-methylthiohydantoin (*Ia*) in toluene at different temperatures and of N-substituted proline thiohydantoins *Ib*, *Ic*, and *Id* at room temperature, are tabulated in Tables I and II. Due to a decreasing ASIS effect the β' and γ' protons in *Ia* are beginning to overlap at higher temperatures. The conformational parameters calculated from the vicinal coupling constants (see Experimental) are summarized in Table III. The endocyclic ring torsion angles are gathered in Table IV.



Ia, R = Me
Ib, R = H
Ic, R = Ph
Id, R = *p*-NO_2Ph



II



III

Temperature Dependence of the ^1H *Spectra of Proline*-N-*methylthiohydantoin* (Ia)

Examination of Table II reveals that the geminal coupling constants of *Ia* are not much influenced by a change of temperature, but that some of the vicinal couplings show a consistent trend at the different temperatures of measurement. This suggests either a change in ring shape or a shift in the ratio of the populations of the two presumed forms N and S. If however the two state model bears physical significance, then to a first approximation the shift in the position of the N $\rightleftharpoons$ S equilibrium (x_N) should be at the origin of the observed effects of change of the temperature.

The analysis of CONFIT of these vicinal coupling constants in terms of conformational parameters (Table III) discloses that the individual N and S conformers are indeed nearly unchanged. However, the ratio of the molfractions of N and S forms changes significantly with temperature.

The population of the major form (N) decreases from $87{\cdot}7 \pm 4{\cdot}4\%$ at $-30°C$ to $77{\cdot}7 \pm 4{\cdot}7$, at $+60°C$, corresponding to equilibrium constants ($K_c = x_N\,x_S$) of $7{\cdot}1 \pm 2{\cdot}2$ ($-30°C$) and $3{\cdot}5 \pm 1{\cdot}0$ ($+60°C$). To the best of our

knowledge this is the first experimental evidence of the kind implicating the validity of the two-state approximation in solution.

Comparison of the Proline Ring Conformation in the N-*Methylthiohydantoin and* N-*Methylhydantoin*

The pseudorotational parameters for the S form are different in the proline N-Me-hydantoin as compared to the N-Me-thiohydantoin. In the former a $\chi_5^N = 20°$ and $\chi_5^S = 22°$ was observed[10]. For proline N-Me-thiohydantoin (*Ia*) we now (Table IV) note at 20°C a $\chi_5^N = 22°$ and $\chi_5^S = 30°$. This is not unexpected in view of the replacement of the N_1 C=O fragment by the N_1 C=S fragment. It is known that the rotational barrier in thioamides is about 3 kcal mol higher[12-14], a fact that has been explained by a greater contribution of the dipolar mesomeric form *II* in the thioamide. In agreement to this, a deviation from planarity of an amide moiety is relatively easy[15-17] as compared to a thioamide. In the latter, the bridgehead nitrogen (N1) possesses more sp^2 character, i.e. a higher resistance to an out-of-plane deformation and, as far as the canonical form *III* (vide infra) is dominant, the thiohydantoin ring becomes flatter. The annelation thus requires a larger χ_5 value. The experimental fact that the increase of χ_5 is pronounced only in the S form remains yet unexplained.

The Effect of N-3 *Substituents on the Pyrrolidine Ring Conformation*

Inspection of the vicinal coupling constants (Table II) of *I* (R = CH_3, H, Ph, *p*-NO_2Ph) does reveal small variations. Translation of these into conformational

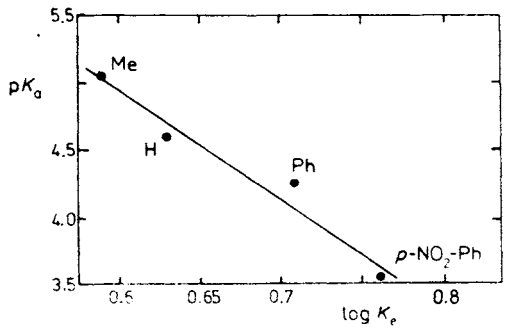


FIG. 1

Graphical representation of the correlation between the pK_a values of *para* substituted anilines and the N⇌S equilibrium constant (log K_e) of the pyrrolidine ring in substituted thiohydantoins *Ia–d*

parameters using the two-state model (see Experimental) shows (Tables III and IV) that the individual N and S forms are quite the same in the series. The molfraction of the N form tends to increase with increasing electronegativity of the N3 substituent (R). However, the effects are small.

Since the aromatic rings in *Ic* and *Id* take an orthogonal position relative to the plane of the hydantoin ring[18], no mesomeric overlap, but only inductive effects of the phenyl and *p*-nitrophenyl groups must be considered.

The pK_a values of *para*-substituted anilines with R = Me, H, Ph (ref.[19]) and *p*-NO_2Ph (ref.[20]) are a measure of the availability of the nitrogen lone pair. In Fig. 1 we related these pK_a values to the corresponding equilibrium constants $K_c = \chi_N/\chi_S$ of the N ⇆ S equilibrium of the pyrrolidine ring in the substituted thiohydantoin series (*I*) in a pK_a logK_c plot. Although the present K_c values have relatively large errors, the obtained regression looks satisfactory ($R^2 = 0{\cdot}97$).

These results are easily rationalized in terms of mesomeric structure *III*. The relative importance of the latter is consistent with the crystallographic study of Walker et al.[21] on thiohydantoin, which reveals short bonds for N1 C2 (1·331 Å) and N3 C4 (1·350 Å), but a long bond for C2 N3 (1·483 Å). Previously, Brown et al.[22] also concluded, from a comparative NMR study of rotation barriers in thioureas and acylated thioureas, the high contribution of the canonical forms corresponding to *III*. As the availability of the N3-lone pair electrons decreases in the direction *Ia* → *Ib* → *Ic* → *Id*, the N3 C4 amide fragment receives less double bond character (while the thioamide delocalization is not affected). This results in an increasing puckering of the hydantoin ring and thus a lower χ_5 value of the pyrrolidine ring, with increasing electronegativity of the substituent. Therefore, the N form, with its intrinsically lower χ_5-value by c. 10° (vide supra) becomes more and more important in the *Ia* → *Ib* → *Ic* → *Id* direction.

CONCLUSION

The two states (N and S) of the pyrrolidine ring in N-methylthiohydantoin preserve their conformational identity as the temperature is raised from −30° to +60°C. By the same their relative populations tend to equalize, typical of an enthalpically controlled equilibrium. It is also shown that in proline hydantoins the small effects of substitution in the hydantoin ring (thio-, or N3-substitution) on the conformation of the pyrrolidine ring can be rationalized as the effects on the "weight" of those resonance structures that govern the flatness of the (thio)amide bond and the hydantoin ring.

Acknowledgement is made to the "Fonds voor Kollektief en Fundamenteel Onderzoek" for financial support in the purchase of our NMR *instrumentation.*

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