

THE STEREOCHEMISTRY AND CONFORMATION OF THE DIASTEREOMERS OF TETRAHYDROTHIAMIN

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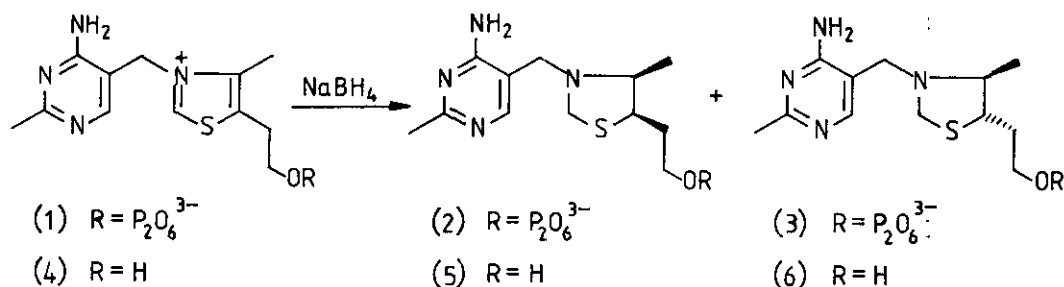
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Abstract—The major isomer of tetrahydrothiamin produced by NaBH_4 reduction of thiamin is shown by ^1H n.m.r. to be cis (5) and its conformation is predominantly (5a) which should be a major factor in the strong inhibition shown by its pyrophosphate (2) towards the pyruvate dehydrogenase complex of E.coli.

Thiamin pyrophosphate (1) is an important coenzyme involved in the enzymic cleavage of carbon-carbon bonds adjacent to carbonyl groups¹. The mechanisms of these reactions involve formation of a carbanion at C-2 of the thiazole ring². It has been found that a racemic mixture of the diastereomers, (2) and (3), of tetrahydrothiamin pyrophosphate (which are catalytically inactive analogues since C-2 is now sp^3 hybridized) is a potent inhibitor of a number of thiamin pyrophosphate-requiring enzymes such as pyruvate decarboxylase³ and transketolase⁴. We have found that tetrahydrothiamin pyrophosphate is a powerful inhibitor of the pyruvate dehydrogenase complex of E.coli and that the different isomers have strikingly different binding constants⁵.

The isomers (2) and (3) can be obtained by pyrophosphorylation of (5) and (6) which are the products of reduction of thiamin (4) with NaBH_4 . However it has not until now been determined which reduction product is cis (5) and which is trans (6). In view of the biological results it was of great interest to know (i) the stereochemistry of the isomers of tetrahydrothiamin and (ii) their conformation in solution, in order to deduce information about the active site of the enzyme. Presented in this paper are the results of a ^1H n.m.r. investigation which proves the stereochemistry of the major isomer to be cis as in (5) and gives a clear indication of its preferred conformation.



only one enantiomer shown

SEPARATION OF THE DIASTEREOMERS OF TETRAHYDROTHIAMIN

Thiamin was reduced with NaBH_4 and the major product (m.p. 147–148°C) purified by repeated recrystallization as described by Clarke and Sykes⁶; λ_{max} (MeOH) 232, 273 ; (0.1M HCl) 243 nm. The

minor isomer was obtained by h.p.l.c. of the mixture on a Varian Micropak MCH-10 (octadecylsilane /silica) column (0.4 x 30 cm) eluting with 40% (v/v) methanol in water. The minor product was eluted first. Seven runs of 6 nmol each gave sufficient of the minor isomer for the n.m.r. experiments. It was about 93% pure by n.m.r. and h.p.l.c..

Table 1. ^1H n.m.r. of the isomers of tetrahydrothiamin

Proton	Major Isomer (5) ^a	Minor Isomer (6) ^b
6	coupling constants Hz ^c	6 coupling constants Hz ^c
Ar-H	7.93 s	7.94 s
H-2a	4.20 d 9.5(H-2b)	3.93 d 9.5(H-2b)
H-2b	3.83 d 9.5(H-2a)	3.88 d 9.5(H-2a)
H-5	3.79 ddd 10.0(H-6b), 5.5(H-4), 4.5(H-6a)	3.29 ddd 10.8(H-6b), 7.5(H-4), 3.5(H-6a)
H-7a	3.70 ddd 10.7(H-7b), 7.2(H-6a), 5.1(H-6b)	3.76 ddd 11.1(H-7b), 6.8(H-6a), 5.1(H-6b)
H-7b	3.59 ddd 10.7(H-7a), 7.1(H-6a, 6b)	3.64 m obscured
H-9a	3.63 s	3.74 d 13.9(H-9b)
H-9b		3.65 d 13.9(H-9a)
H-4	3.42 dq 6.2(H-5, H-8)	2.95 dq 6.8(H-5, H-8)
ArCH ₃	2.44 s	2.44 s
H-6a	2.00 dddd 13.3(6b), 7.4(7a, 7b), 4.3(H-5)	2.13 dddd 13.8(6b), 7.3(7a, 7b), 5.2(H-5)
H-6b	1.76 dddd 13.3(6a), 10.4(H-5), 6.8(7b), 5.4(7a)	1.73 dddd 13.8(6a), 10.8(H-5), 5.9(7b), 5.2(7a)
H-8	1.10 d 6.8(H-4)	1.34 d 6.5(H-4)

^a Recorded in CD₃OD solution at both 400MHz and 250 MHz.

^b Recorded in D₂O solution at both 400MHz and 250 MHz.

^c As measured from the spectrum. Inaccuracies may occur where overlapping peaks are not resolved.

STEREOCHEMISTRY OF THE DIASTEREOMERS

The ^1H n.m.r. of the two isomers measured at 400 and 250 MHz are given in table 1. The assignments follow from the chemical shifts and coupling patterns and were rigorously confirmed by decoupling experiments. To establish its stereochemistry, n.O.e. experiments were performed on the major isomer. The enhancements observed are shown in table 2. The n.O.e.'s between H-4 and H-5 and from H-8 to H-6a and H-6b clearly show that the substituents at C-4 and C-5 are cis to one another and so the major isomer is (5). To confirm this assignment the corresponding n.O.e. experiments were performed on the signals due to the minor isomer in the unresolved mixture of (5) and (6) (table 2)⁷. The n.O.e.'s between H-8 and H-5 and between H-4 and H-6 fully prove that this isomer has the trans stereochemistry (6).

Table 2. N.O.e.'s observed for the isomers of tetrahydrothiamin.

Major Isomer (5) ^d		Minor Isomer (6) ^e	
Irradiated	n.O.e.'s observed	Irradiated	n.O.e.'s observed
H-8	H-2a, H-4, H-6a, H-6b	H-8	H-4, H-5
H-6a	H-6b, H-8	H-6a	H-4, H-5
H-4	H-5, H-8, H-9	H-4	H-6a, H-8
H-5	H-4	H-5	H-6b, H-8
H-9	H-2b, H-4, H-5, Ar-H		
H-2a	H-2b, H-8		

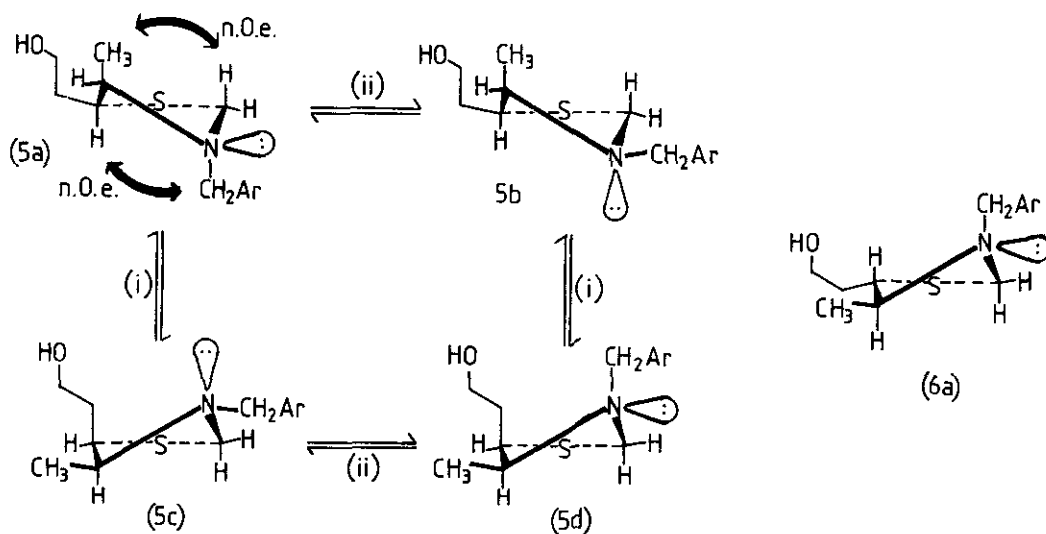
^d In CD₃OD solution at 400 MHz.

^e In a D₂O solution of the mixture of isomers at 250 MHz⁷.

It has been observed that in simple thiazolidines the coupling constant between H-4 and H-5 is smaller if they are *cis* than if they are *trans*⁸. In this case also, $J_{H,5}$ for the *cis* isomer (5.5 Hz) is smaller than for the *trans* isomer (7.5 Hz). On chemical grounds it is reasonable that the major product of the reduction of thiamin is *cis* as the hydride has been delivered from the less hindered side.

CONFORMATION OF (5) AND (6)

There has been considerable interest in the conformations of thiazolidine rings^{8,9}. These studies conclude that the most stable conformation is close to a half chair with N-3 and C-4 on opposite sides of the plane through the other three atoms. Thus there are four possible conformations of the thiazolidine ring of (5), viz (5a-d); they are generated by (i) flipping of the ring and (ii) inversion at the nitrogen atom. As n.o.e.'s were observed from H-8 to H-2a and H-9 to H-5, it can be concluded that (5a) is the major conformation because only in this conformation do the appropriate hydrogens come close to each other (indicated by arrows on the diagram). For other conformations, other n.o.e.'s would be predicted which were not observed and so the time that a molecule spends in these conformations must be small compared with (5a).



Further evidence for an axial substituent on N-3, as in (5a), can be found in the chemical shifts of H-2a and H-2b and the coupling between them. It has been shown that in thiazolidines where the nitrogen lone pair is *trans*-diaxial with a proton on C-2, the signal from that proton is moved upfield and the geminal coupling constant is reduced to ca. 6 Hz¹⁴ but where the nitrogen lone pair is antiperiplanar to the C-S bond the geminal coupling constant is 9-10 Hz^{10,14}. In (5) the coupling between H-2a and H-2b (9.5 Hz) is as expected for the conformation (5a) having an equatorial lone pair on nitrogen. Also the axial proton on C-2 is the downfield one (as proved by the n.o.e.) and it is evidently not shifted upfield by a *trans* diaxial lone pair.

Though energy differences between different conformations of a five-membered ring are usually small, in this case (5a) has a large energetic advantage over the other conformations because the substituent on nitrogen prefers to be axial and in (5d) models show considerable steric compression between this axial substituent and the pseudo-axial hydroxy-ethyl group on C-5. The tendency of

nitrogen substituents to occupy the axial position in 1,3-diheterocycles has been observed often in 6-membered rings¹¹ and recently in 1,3-dimethylimidazolidine also¹². However this seems to be the first time it has been demonstrated in a simple thiazolidine. The effect has been explained both by dipole-dipole repulsion between the lone pairs on the two heteroatoms¹⁰ and by a general anomeric effect in which the nitrogen lone pair donates into the antibonding orbital of the adjacent carbon-heteroatom bond¹¹. It is notable that simpler 2-unsubstituted thiazolidines have $J_{2a,2b} = 9-10 \text{ Hz}$ ^{6,13} and so they presumably also exist with the nitrogen lone pair equatorial.

The coupling constant between H-2a and H-2b in (6) is also 9.5 Hz, suggesting that again the nitrogen substituent is axial. No n.o.e. was observed between H-8 and H-2a or 2b and so it is probable that the major conformation is (6a) though significant amounts of other conformations may be in equilibrium with it.

The conformations deduced for (5) and (6) are for the free base, however addition of $\text{CD}_3\text{CO}_2\text{D}$ to the solution of (5) in CD_3OD caused only small downfield shifts to the protons near N-3 whereas addition of $\text{CF}_3\text{CO}_2\text{D}$ caused larger shifts (up to 0.3 p.p.m.). No coupling constant was materially altered on acidification and a n.o.e. was still observed from H-8 to H-2a; therefore it seems likely that the preferred conformation remains unchanged. Thus it can be assumed that conformation (5a) is the prevailing one at physiological pH.

CONCLUSION

The major product of sodium borohydride reduction of thiamin is the cis tetrahydrothiamin (5) which exists largely in conformation (5a) in solution. As the pyrophosphate of (5) is the isomer which binds more strongly to the pyruvate decarboxylase component of the *E.coli* pyruvate dehydrogenase complex than thiamin pyrophosphate itself⁵, the conformation of (5) is likely to be highly significant in determining the strength of binding of the inhibitor.

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14. Magnitude of the coupling constant only. As is usual with geminal couplings, the sign is negative.

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