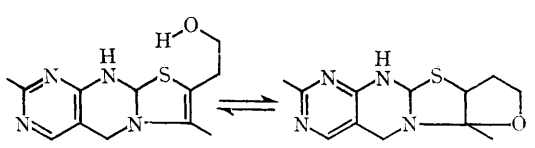
The Functions of the 4-Amino-2-methylpyrimidinyl and 5-2′-Hydroxyethyl Groups in the Nonenzymic Reactions of Thiamine

By G. E. RISINGER,* E. J. BREAUX, and H. H. HSIEH

(*Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana* 70803)

THE model reaction discovered by Mizuhara and Handler[1] has been employed to study the mechanism of thiamine action. Several investigators[2] have noted that thiamine is the superior catalyst for this reaction, and they have traced the increased activity to the presence of the 4-amino-2-methylpyrimidinyl and 5-2′-hydroxyethyl groups of the thiazolium nucleus. The function of these groups, however, is obscure. In an attempt to clarify the roles of these groups, we have observed the n.m.r. spectrum† of thiamine in the presence of two equivalents of methoxide. We observed the spectra of thiamine in both D_2O and methyl alcohol and in both cases the signal at 9·7 p.p.m. (C-2, H) disappears and a new signal (singlet) appears at 1·70 p.p.m. The new signal, however, is absent in the spectra of both *O*-acetylthiamine and thiamine pyrophosphate. In addition, it was discovered that the new signal is temperature dependent. When the temperature is increased from 37 to 50°, a signal appears at 2·12 p.p.m. which develops at the expense of the 1·70 p.p.m. signal, and the system attains equilibrium after *ca.* 70 min. It was also noted that the protons responsible for the 1·70 p.p.m. signal will not exchange in D_2O.

In order to explain these phenomena, we have suggested the following reaction sequence:



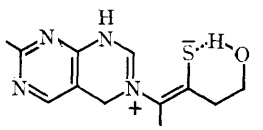
(I) (II)

Initially, thiamine reacts with two equivalents of base to afford (I). (I), *via* the enamine, undergoes cyclization to yield the *NO*-acetal (II). At lower temperatures, the acetal predominates; at higher

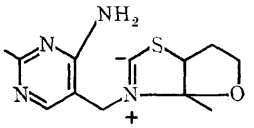
temperatures, the amount of the tricyclic form (I) of thiamine increases.

We have examined the n.m.r. spectra of dihydrothiamine[3] and the $C_{12}H_{16}N_4SO$ compound isolated by Metzler,[4] and have found that both compounds have the characteristic singlet at 1·70 p.p.m. indicating the *NO*-acetal linkage. In addition, the spectra are temperature dependent with a singlet at 2·12 p.p.m. occurring at the expense of the 1·70 p.p.m. signal. Based on these observations we propose that the function of the aminopyrimidyl and 5-2′-hydroxyethyl groups is to preserve the thiazoline ring structure. Without this stabilization, the thiazolium salt would undergo hydrolytic cleavage with loss of the active site at C-2; this ease of hydrolysis, as initially stated by Breslow,[2a] is probably the reason that 3-4′-nitrophenylthiazolium salts are such poor catalysts in the Mizuhara–Handler reaction.

Unfortunately, this Scheme does not clarify the mechanism of the nonenzymic reaction. This reaction may be ring-opening of (II) to afford dihydrothiochrome (I) which affords either the zwitterion, proposed by Breslow, or the sulphydryl anion (III) which has been recently suggested.[2b]



(III)



(IV)

The reaction can occur by the acetal compound (II) undergoing displacement of the pyrimidyl group to yield the zwitterion (IV) which is a possible candidate[5] for the catalytic form of the vitamin.

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† All n.m.r. spectra were determined on a Varian A-60 in D_2O and MeOH containing DSS and tetramethylsilane, respectively.

[1] S. Mitzuhara and P. Handler, *J. Amer. Chem. Soc.*, 1954, **76**, 571.
[2] (a) R. Breslow, *J. Amer. Chem. Soc.*, 1958, **80**, 3719; (b) G. E. Risinger and M. F. Dove, *Chem. and Ind.*, 1965, 510; (c) C. D. May and P. Sykes, *J. Chem. Soc.* (*C*), 1966, 649.
[3] G. E. Bonvicino and D. G. Hennessy, *J. Amer. Chem. Soc.*, 1957, **79**, 6325.
[4] G. E. Maier and D. E. Metzler, *J. Amer. Chem. Soc.*, 1957, **79**, 4386, 6583.
[5] W. H. Hafferl, R. E. Lundin, and L. L. Ingraham, *Biochemistry*, 1964, **3**, 1072.