Studies on the Aminopyrimidinyl Group of Thiamine[1]

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Although 3-(4-amino-2-methyl-pyrimidin-5-yl)methyl-5-ethoxycarbonyl-4-methylthiazolium chloride (**2**) undergoes a rapid C-2 H/D exchange in acidic D_2O, formation of its neutral tricyclic form in basic solution completely inhibits the ability of this thiamine analogue to catalyse the benzoin condensation.

Subsequent to the work of Crosby and Lienhard and as a possible model of the active-site chemistry of thiamine pyrophosphate (TPP),[2] studies on thiamine have focused on its efficient catalysis of benzoin-type condensations in basic non-aqueous solution.[3] Maier and Metzler reported that an interesting feature of the chemistry of thiamine under these conditions in basic non-aqueous solution is its conversion into the neutral tricyclic form (**1**) (Scheme 1).[4] Important properties of this tricyclic species are its stability (relative to the ionic forms of thiamine) in hydrophobic solvents such as ethanol and butanol,[4] and the lability of its C-9a proton, as evidenced by the ease with which the tricyclic form is oxidized by I_2 and oxygen to thiochrome.[5,6] Although Metzler argued

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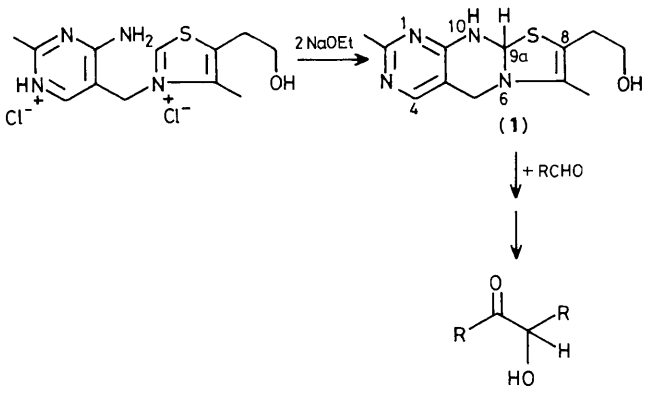
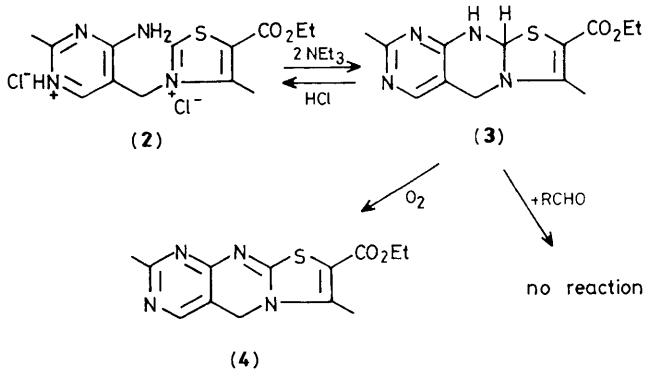
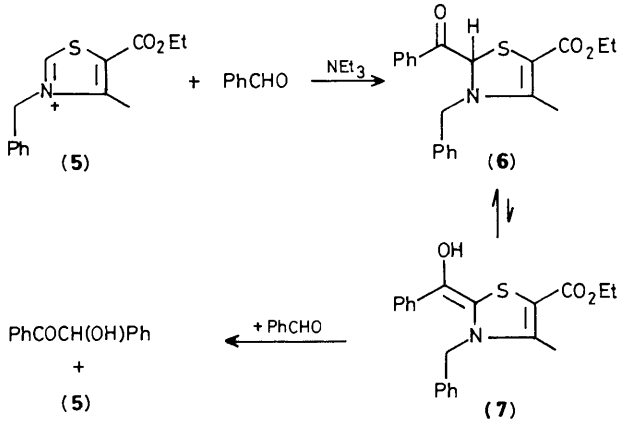


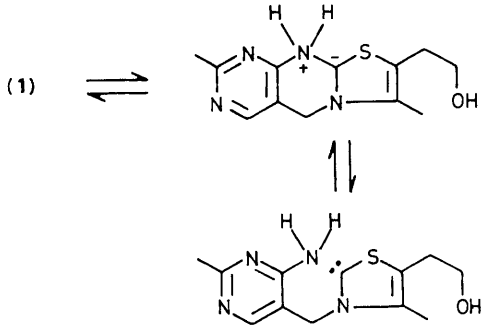
Scheme 1



Scheme 2



Scheme 3



Scheme 4

that this chemistry of thiamine must be an important part of the chemistry of TPP at an enzyme active-site, there had been no obvious rationale for considering that the aminopyrimidinyl group could have any effect, other than inductive, on the reactivity of the C-2 position of thiamine.[7] We now report studies on a thiazolium salt related to thiamine in which the *N*-(aminopyrimidinyl) group has a dramatic effect on the chemistry of the thiazolium ring.

When 3-(4-amino-2-methylpyrimidin-5-yl)methyl-5-ethoxycarbonyl-4-methylthiazolium chloride hydrochloride (**2**)‡ was treated in refluxing acetone with two equivalents of NEt_3 under nitrogen, the resultant tricyclic form (**3**) was isolated in quantitative yield (Scheme 2).§¶ This tricyclic species can be easily converted into the salt (**2**) with dry HCl, and oxidized to the resultant thiochrome derivative (**4**) in refluxing acetone in the presence of oxygen. Although the salt (**2**) undergoes a rapid H/D exchange at C-2 in acidic D_2O [$k_{ex.}$ of (**2**) is 80-fold larger than $k_{ex.}$ of thiamine], when (**2**) was refluxed in methanol with two equivalents of NEt_3 in the presence of an excess of benzaldehyde there was no detectable formation of benzoin, and the tricyclic species (**3**) was isolated in quantitative yield.

This chemistry of thiazolium salt (**2**) is in contrast to the chemistry of the corresponding 5-ethoxycarbonyl-4-methyl-3-phenylmethylthiazolium bromide (**5**). When the salt (**5**) was refluxed in methanol containing two equivalents of triethylamine and an excess of benzaldehyde, the 2-benzoylthiazoline (**6**) was isolated in 70% yield and benzoin [produced *via* the C-2α reactivity of the 'active aldehyde' (**7**)] was detected by n.m.r. and t.l.c. analysis of the reaction mixture (Scheme 3). This result demonstrates that although the C-2 deprotonated intermediates of the *N*-alkyl salts of 5-ethoxycarbonyl-4-methylthiazole form readily in basic non-aqueous solution, formation of the tricyclic species (**3**) completely prevents formation of the C-2 deprotonated intermediate of salt (**2**).

‡ Prepared by the condensation of 5-ethoxycarbonyl-4-methylthiazole and 4-amino-5-chloromethyl-2-methylpyrimidinium hydrochloride in propan-1-ol at 104 °C for 5 h. The product was precipitated by the addition of ethanol and recrystallized from hot ethanol. M.p. 220 °C.

§ All products gave elemental analyses, mass spectra, and ^1H n.m.r. spectra consistent with the proposed structures.

¶ Product (**3**) was precipitated by the addition of deoxygenated water under an inert atmosphere. ^1H n.m.r. ([$^2H_6$]acetone): δ 1.3 (t, 3H, *J* 7 Hz, OCH_2CH_3), 2.25 (s, 3H, thiaz-CH_3), 2.45 (s, 3H, pyr-CH_3), 4.20 (q, 2H, *J* 7 Hz, OCH_2), 4.45 (s, 2H, pyr-CH_2), 6.40 (s, 1H, orthoester-C*H*), 6.9 (br.s, 1H, exchangeable NH), 7.95 (s, 1H, pyr-H).

This is in contrast to the chemistry of the tricyclic form of thiamine. When the tricyclic form of thiamine is generated at 0 °C in basic ethanol (thiamine-HCl, 2 equiv. NaOEt)[4] and benzaldehyde (10 equiv.) is added, there is a rapid accumulation of an intermediate in high concentrations which gives rise to benzoin which, after five hours, can be isolated in 250% yield (based on initial thiamine), *i.e.* the reaction is catalytic (Scheme 1). In this system, then, it is unlikely that conversion of the tricyclic form of thiamine into the C-2 deprotonated thiamine intermediate is the rate-limiting step of the thiamine-catalysed benzoin condensation.

This chemistry demonstrates that an intramolecular cyclization can have a profound effect on the chemical reactivity of a thiazolium ring. In this case, the stability of the tricyclic form (**3**) completely inhibits the benzoin condensation in basic non-aqueous solution. Based on our results it appears that thiamine has evolved such that there is a

very delicate balance between the nucleophilicity of the aminopyrimidnyl group toward the C-2 position of the thiamine thiazolium ring, and the leaving group potential of the aminopyrimidinyl moiety of the tricyclic form of thiamine; this is of course one requirement for a nucleophilic catalyst. It may be that the absolute requirement for the aminopyrimidinyl group of thiamine pyrophosphate in enzymic systems is a result of its ability to function as a novel, intramolecular nucleophilic catalyst (*e.g.* Scheme 4).‖

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‖ The sequence in Scheme 4 is the reverse sequence for the one proposed by Hopmann *et al.* (ref. 8a) to account for formation of the tricyclic form of thiamine from the C-2 deprotonated intermediate. We prefer to use the carbene resonance structure (ref. 8b) because of the similarity between this sequence and the base-induced decomposition of chloroform to dichlorocarbene (ref. 8c).

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