Kinetics and Thermodynamics of the Structural Transformation of Thiamine and its Analogues in Aqueous Media. Part 4.† The Case of Sulphamoylthiamine

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In sulphamoylthiamine (ST) one of the two amino-group protons at the pyrimidine ring of thiamine is replaced by a sulphamoyl group, which should prevent the formation of a yellow form analogous to that of thiamine. Nevertheless, in basic media, ST changes into a yellow form with the loss of two protons (average pK 11.59). The rate-limiting step of this transformation is the intramolecular nucleophilic attack at the 2-site by the 7'-amine to yield σ-adduct (**D'**) with a second-order rate constant k_{14} = 66 dm^3 mol^{-1} s^{-1}. Adduct (**D'**) is in turn transformed into (**J'**)$^-$ with a second-order rate constant k_{45} = 7.90 × 10^6 dm^3 mol^{-1} s^{-1}. Species (**J'**)$^-$ yields ST thiolate (**C'**)$^-$ *via* intramolecular σ-adduct (**D'**), cation (**A'**)$^+$ and pseudo-base (**B'**), the formation of which is the rate-limiting step of the structural transformation of ST in basic media (second order rate constant k_{34} = 10.80 dm^3 mol^{-1} s^{-1}). Although different from those values measured for thiamine, these rates and equilibrium constants show that the reactions involved in the structural transformations of ST and thiamine occur essentially in the same way. However, the secondary amine in (**D**) is replaced by a tertiary amine in (**D'**) which can be deprotonated at the 2-position to yield anion (**D'**)$^-$ which, by ring-opening, can produce a resonance-stabilized carbene species resembling Breslow's biocatalyst (**A**)$_{-H}$. The ^{13}C NMR spectra of (**J**)$^-$ and (**J'**)$^-$ seem to confirm this hypothesis which is consistent with the involvement of species (**D**)$^-$ in the structural transformations of thiamine in basic media.

Thiamine (vitamin B_1) is responsible for many reactions involved in carbohydrate metabolism in the tricarboxylic acid cycle[2] (*e.g.* pyruvate decarboxylation).[3] Beslow showed in the late 1950s that the 2-H in thiamine exchanges with deuterons in heavy water. He ascribed this lability to the direct deprotonation of C-2 to yield the resonance-stabilized carbene species (**A**)$_{-H}$.[4]

In our preceding papers, we proposed a general mechanism for the structural transformations of thiamine in aqueous media.

Mechanism

$$(\mathbf{A})^+ + OH^- \rightleftharpoons (\mathbf{B}) \quad (1)$$

$$(\mathbf{B}) + H^+ \rightleftharpoons (\mathbf{A})^+ \quad (2)$$

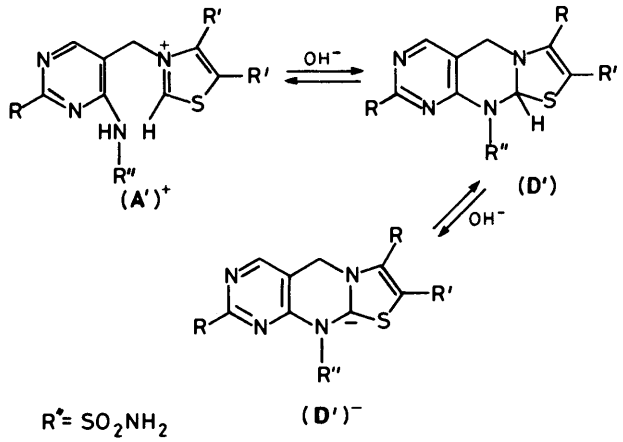
$$(\mathbf{B}) + OH^- \rightleftharpoons (\mathbf{C})^- \quad (3)$$

$$(\mathbf{A})^+ + OH^- \rightleftharpoons (\mathbf{D}) \quad (4)$$

$$(\mathbf{D}) + OH^- \rightleftharpoons (\mathbf{J})^- \quad (5)$$

Without rejecting direct deprotonation of the thiamine C-2 position, we have estimated that the lability of 2-H in basic media can reasonably take place *via* a new anionic structure (**D**)$^-$ formed from the deprotonation of σ-adduct (**D**). Recently, Washbaugh and Jencks by performing experiments in acidic media on (**A**)$^+$ and (**A**)H^{2+} (thiamine protonated at 1'-N), estimated that the deprotonation pK values of thiamine and some other thiazolium salts are *ca.* 18. They also proposed that the aminopyrimidinyl group does not provide a significant intramolecular catalysis of non-enzymic 2-H removal from the vitamin.[5] These results confirm our proposals concerning the high deprotonation pK value of thiamine.[1c] They cannot, however, explain our findings in basic and very basic media. Therefore, in order to confirm the eventual involvement of the (**D**)$^-$ species in the transformation of the thiamine-yellow form, we now report a physicochemical analysis of the structural transformations of sulphamoylthiamine (ST) in basic media.[6,‡]

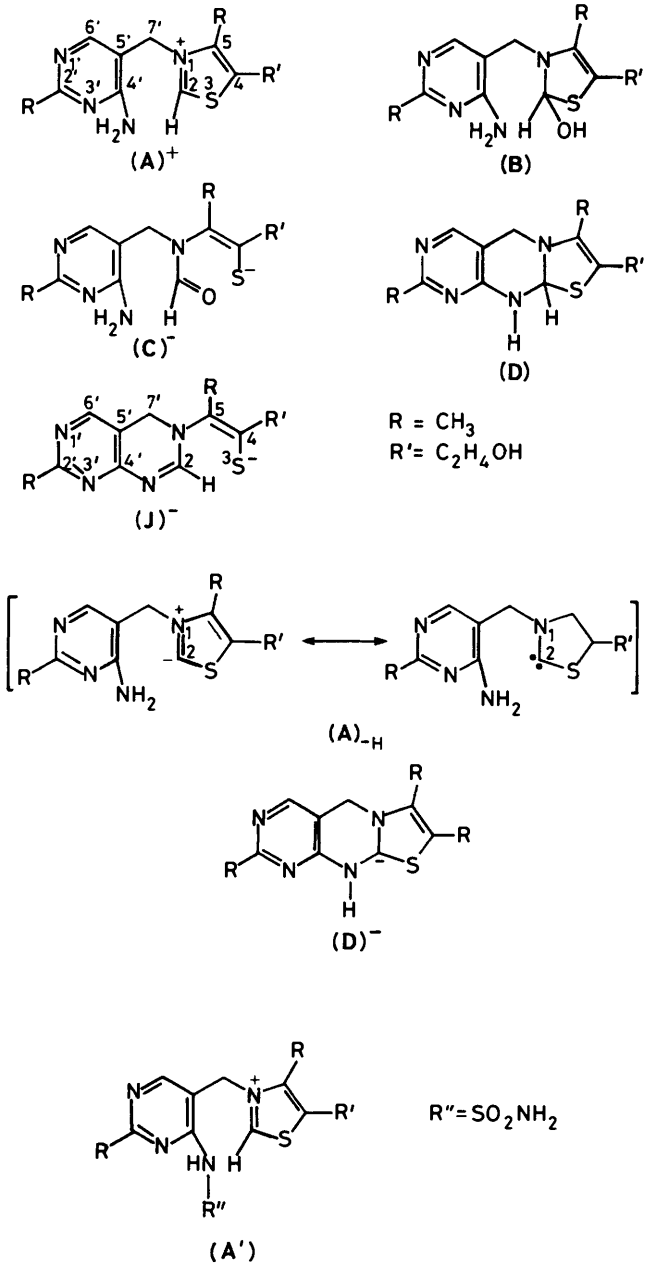
The formation of the thiamine-yellow form (**J**)$^-$ requires the loss of the two amino protons.[8] In ST one of these protons is replaced by a sulphamoyl group which is known to be stable in basic media.[6] Such a thiamine analogue cannot yield a yellow form analogous to (**J**)$^-$. We thus hoped that ST cation (**A'**)$^+$ would give ST σ-adduct (**D'**) and eventually the ST carbanion (**D'**)$^-$, according to Scheme 1.



Scheme 1.

† For part 3, see ref. 1(*c*).

‡ Although the sulphamoyl group is terminated by a primary amine which may play the role of a nucleophile (and, therefore, perturb the formation of the expected σ-adduct), sulphamoylation of thiamine was preferred to methylation, because it is specific to primary and secondary amines only.[6] In spite of the fact that Me-*N*-7'-thiamine is reported in the literature[4] methylation occurs chiefly at the 1'-N leading to Me-*N*-1'-thiamine[7] which is of no use for our purpose.



Experimental

Sulphamoylthiamine was provided by Drs. M. Hedayatullah and J. C. Hugueny.[6] NaOH and HCl (Merck Titrisol), KCl (Merck suprapur), CD_3OD (Aldrich Gold Label) and buffers (Beckman NBS) were used without further purification. Water was distilled twice under argon.

Stock Solutions.—Fresh solutions of ST were used at concentrations c ranging from 5×10^{-5} to 4×10^{-2} mol dm^{-3} for kinetics and from 0.1–0.4 mol dm^{-3} for NMR spectroscopy.

pH Measurements.—pH values were measured with a Radiometer pH-meter equipped with a 'Metrohm E.A. 125' combined electrode. Buffers used for pH standardization were pH 6.86 and 10.01 NBS standards (Beckman).[1]

Slow Kinetic Measurements.—For the $(J') \longrightarrow (C')^-$ transformation, the neutral solutions of ST ($5–10 \times 10^{-5}$ mol dm^{-3}) were perturbed by injecting microvolumes of NaOH according to previously described methods.[1] Kinetic measurements were performed on a nitrogen-purged Cary C 210 spectrophotometer. The sample cell was thermostatted at (25 ± 0.5) °C.

Stopped Flow.—Kinetic measurements were performed under argon on a Canterbury Hightech stopped-flow spectrophotometer equipped with a thermostatted bath held at (25 ± 0.5) °C and with a Hewlett–Packard memory unit. Output voltage was adjusted to 5 V for zero absorbance. Equal volumes of solutions of NaOH ($1–40 \times 10^{-2}$ mol dm^{-3}) and of neutral ST ($3–4 \times 10^{-4}$ mol dm^{-3}) were simultaneously injected into the mixing chamber (mixing time < 5 ms). Final $[OH^-]$ values were deduced from the diluted NaOH/ST mixture by considering the need for 2 equiv. of OH^- to transform $(A')^+$ into $(J')^-$. The final (post-perturbation) ionic strength was adjusted to 0.2 mol dm^{-3} with KCl.

T-Jump Kinetics.—A Messanlagen und Studiengesellschaft *T*-jump spectrophotometer equipped with an external reservoir thermostatted at (2 ± 0.5) °C was used for the kinetic analysis as described previously for thiamine.[1c] In these experiments, the ST solutions, in concentrations ranging from $1–4 \times 10^{-3}$ mol dm^{-3}, were prepared in 0.1 mol dm^3 phosphate buffers, the ionic strengths of which were adjusted to 0.5 mol dm^{-3} with KCl. The *T*-jump was performed by discharging a 0.05 μF condenser charged at 20 kV through a solution of $(J')^-$ as described for thiamine.[1] The temperature was raised by *ca.* 4 °C over *ca.* 5 μs. The pH and initial temperature were measured in the apparatus reservoir as described elsewhere.[1c] The signals were acquired on a PDP 11 computer.

^{13}C NMR Measurements.—^{13}C NMR measurements were performed on a Bruker 200 MHz (50 MHz for ^{13}C) Fourier transform NMR spectrometer. The spectrum of $(A')H^{2+}$ [$(A')^+$ protonated at 1'-N)] was acquired in 60% H_2O–40% CD_3OD by 131 scans and a spectral bandwidth of 20 kHz. Slight proton decoupling was used in order to avoid heating the anionic thiamine and ST solutions. $(J)^-$ and $(J')^-$ were generated at 263 K by fast injections of the required amounts of 10 mol dm^{-3} NaOH into the $(A)H^{2+}$ and $(A')H^{2+}$ solutions, respectively. At this temperature the lifetimes of $(J)^-$ and $(J')^-$ in the alcoholic basic medium were *ca.* 3–4 h. It should be noted, however, that $(J')^-$ is less soluble than $(J)^-$ in the basic alcoholic medium. The spectra of $(J)^-$ and $(J')^-$ were recorded with slight proton decoupling with 600 and 556 scans, respectively, and a spectral bandwidth of 20 kHz. The ^{13}C DEPT spectra were recorded with a spectral bandwidth of 10 kHz by use of the appropriate sequence provided by Bruker using 600 scans, a pulse time of 168 μs and an acquisition time of 20 s for $(J)^-$ and 444 scans, a pulse time of 252 μs, and an acquisition time of 20 s for $(J')^-$.

Signal Analysis.—All the experimental signals were analysed by the semi-log method with least-squares adjustments.[1] They were all pure exponentials.

Results and Discussion

When a neutral solution of $(A')^+$ is rendered strongly basic (pH > 11), it rapidly becomes yellow. This colouration slowly fades to reform the colourless solution. The UV spectra of these three ST species are close to the spectra of $(A)^+$, $(J)^-$, and $(C)^-$ (ref. 1 and 8) (Figure 1).

Kinetic Processes.—When a neutral solution of $(A')^+$ is rendered very basic (pH > 11), two kinetic processes are observed in the 300–400 nm range. Initially observed is a fast increase of absorbance followed by a slow exponential decrease of absorbance (Figure 2). The fast phenomenon is time-resolved

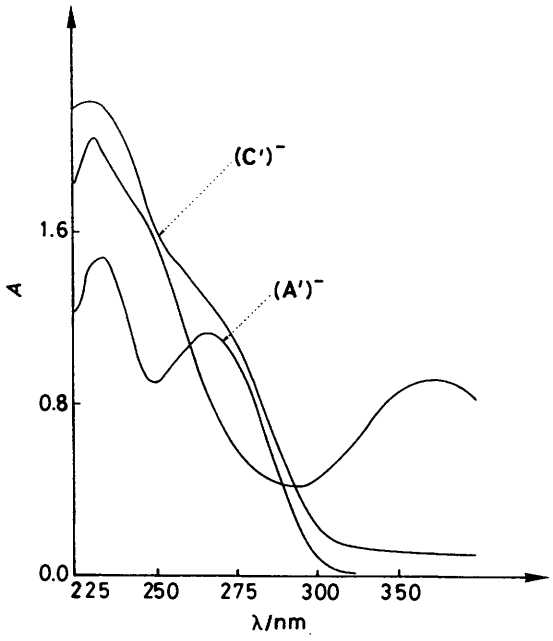


Figure 1. Absorbance spectra of $(A')^+$, $(C')^-$, and $(J')^-$ recorded at pH 7.4 and 12.5, respectively. The spectrum of $(J')^-$ was recorded over 1 min following a fast basification of the $(A')^+$ solution, and that of $(C')^-$ was recorded 2 h later for an ST concentration $c = 1.6 \times 10^{-4}$ mol dm^{-3}.

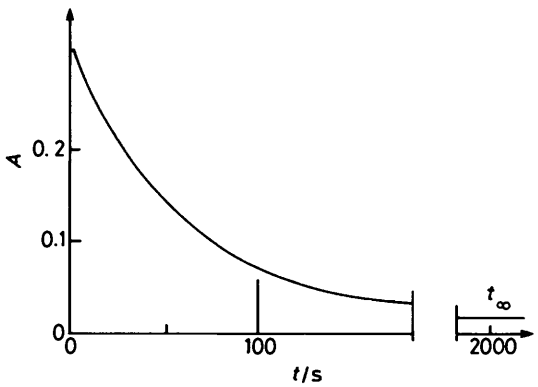


Figure 2. Absorbance change at 339 nm for a solution of sulphamoylthiamine when subjected to a fast pH jump from neutral (pH *ca.* 7) to very basic (pH *ca.* 11.64) at 25 °C and an ionic strength of 0.2 mol dm^{-3} ($c = 1.2 \times 10^{-4}$ mol dm^{-3}).

by the stopped-flow technique and occurs as a fast exponential increase of absorbance, the amplitude of which becomes pH independent above pH 12.5. Thus, $(A')^+$ is transformed into an ST thiolate, $(C')^-$. Moreover, when a solution of the yellow form [obtained by a fast basification of a solution of $(A')^+$] is exposed to a fast *T*-jump, a relaxation mode, which occurs as an increase of absorbance in the 300–360 nm zone and in the 10^{-4} second range, is observed. The amplitude of this fast phenomenon depends on the pH and on the ST concentration. It becomes extremely fast above pH 12 where it cannot be further analysed by the *T*-jump technique.

The Equilibrium Constant of the $(A')^+$ *ST Yellow-species Formation.*—In mildly basic media, in which we only observe the formation of pseudobase (**B**),[1] the kinetics of sulphamoylthiamine should resemble those of thiamine itself.

$$(A')^+ + OH^- \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} (B') \qquad (6)$$

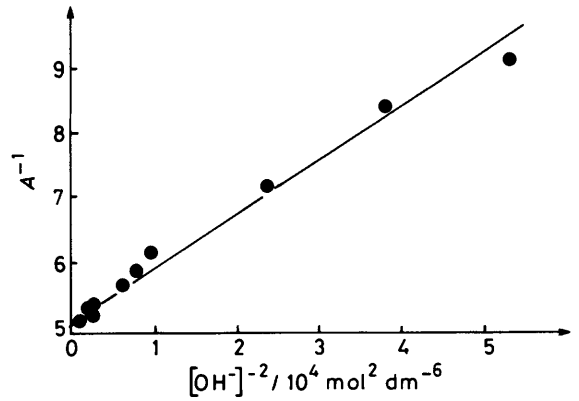
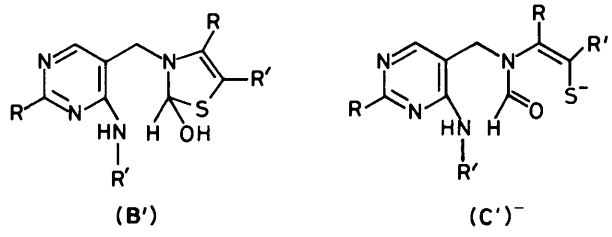


Figure 3. Plot of $1/A$ *vs.* $[OH^-]^{-2}$; intercept, (5.15 ± 0.85); slope, (7.90 ± 0.70) × 10^{-5} mol dm^{-3}; *r*, 0.993 34.



$$(B') + OH^- \rightleftharpoons (C')^- \qquad (7)$$

However, if in basic media $(A')^+$ yields σ-adduct (**D'**), only reaction (8) [the $(A')^+ \longrightarrow (D')$ transformation] will occur with 1 equiv. of OH^- (Scheme 1).

$$(A')^+ + OH^- \underset{k_{41}}{\overset{k_{14}}{\rightleftharpoons}} (D') \qquad (8)$$

In this case the equilibrium constant for the transformation $(A') \longrightarrow (D')$ can be measured by spectrophotometric techniques. Since the molecular extinction coefficients of $(A')^+$ and $(C')^-$ at 340 nm are negligible compared with that of the yellow species (ε), for an optical path of 1 cm, the Benesi–Hildebrand relation can be expressed as equation (9), where *A*

$$1/A = 1/c\varepsilon + (K_{3b}/c\varepsilon)(1/[OH^-]) \qquad (9)$$

is the amplitude of the fast increase of absorbance (Figure 2), $K_{3b} = [OH^-][(A')^+]/[(D')]$ and *c* is the analytical concentration of thiamine. The experimental data do not correctly fit equation (9). Therefore, the transformation of $(A')^+$ into the ST yellow form does not occur with 1 equiv. of OH^-. Thus the yellow form of ST is not (**D'**). If we now assume that the formation of ST yellow form requires 2 equiv. of OH^-, reactions (8) and (10), the Benesi–Hildebrand relation can be expressed

$$(D') + OH^- \underset{k_{54}}{\overset{k_{45}}{\rightleftharpoons}} (J')^- \qquad (10)$$

as equation (11), where $\bar{K}_b = [OH^-]^2[(A')^+]/[(J')^-] =$

$$1/A = 1/c\varepsilon + (\bar{K}_b/c\varepsilon)(1/[OH^-]^2) \qquad (11)$$

$K_{3b}K_{4b}$ is the overall equilibrium constant of the structural transformation of ST into its yellow form (**J'**) and $K_{4b} = [OH^-][(D)]/(J')]$.

A linear least-squares regression of the data *vs.* equation (11) (Figure 3) gave: $p\bar{K} = 11.59 \pm 0.02$. Thus, the formation of the yellow form of ST requires 2 equiv. of OH^-.

Mechanistic Approach.—Since all the kinetic phenomena observed were purely exponential, they were dealt with as

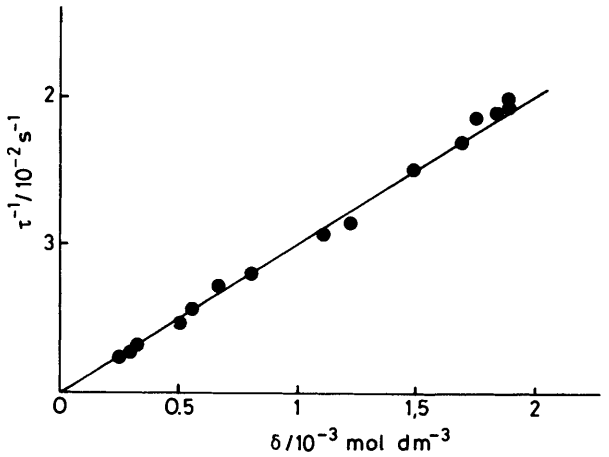


Figure 4. Plot of τ^{-1} *vs.* δ; intercept, $-(8 \pm 36) \times 10^{-4}$ s^{-1}; slope, (10.80 ± 0.50) dm^3 mol^{-1} s^{-1}; *r*, 0.997 89, where $\delta = K_{3b}\bar{K}[OH^-]/(\bar{K}K_{3b} + K_{3b}[OH^-]^2 + \bar{K}[OH^-])$.

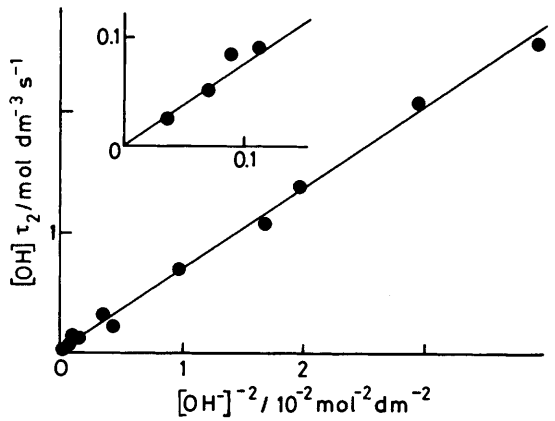


Figure 5. Plot of $[OH^-]\tau_2^{-1}$ *vs.* $[OH^-]^2$; intercept, $(8 \pm 17) \times 10^{-3}$ s^{-1}; slope, (66 ± 4) dm^3 mol^{-1} s^{-1}; *r*, 0.998 77.

relaxation processes.[9] Below pH 11.6–11.7, the reciprocal relaxation times of the slow decrease of absorbance of Figure 2 are proportional to pH, while above pH 11.6–11.7 they become inversely proportional to pH. Thus, the transformation of the ST yellow form into $(C')^-$ resembles that of $(A)^+$ into $(C)^-$ in the same media.[1,8] We can, therefore, assume that $(J')^-$ will yield $(C')^-$ *via* (D'), (A'), and (B'). Moreover, the requirement of 2 equiv. OH^- for the transformation $(A')^+ \longrightarrow (J')^-$ allows the measurement of $pK_3 = 12.70 \pm 0.05$ and $pK_4 = 10.50 \pm 0.05$[1] instead of 10.90 and 12.30, respectively, for thiamine.[1] Thus the sulphamoylation of the amino group encourages the formation of $(J')^-$ at the expense of the σ-adduct (D').

The Slow Kinetic Process.—The slow kinetic phenomenon of Figure 2 should be ascribed to the slowest reaction of the structural transformations of ST in basic media, which in the case of thiamine is the hydroxylation of $(A)^+$ into pseudobase (B). This slow relaxation mode can, therefore, be ascribed to reaction (6), the reciprocal relaxation-time equation of which is expressed as equation (12).[1]

$$\tau^{-1} = k_{34}K_{3b}\bar{K}[OH^-]/(\bar{K}K_{3b} + K_{3b}[OH^-]^2 + \bar{K}[OH^-]) \quad (12)$$

A linear least-squares regression of the data employing equation (12) (Figure 4) gave, at 25 °C and 0.2 mol dm^{-3} ionic strength, $k_{34} = (10.80 \pm 0.50)$ dm^3 mol^{-1} s^{-1} which is almost

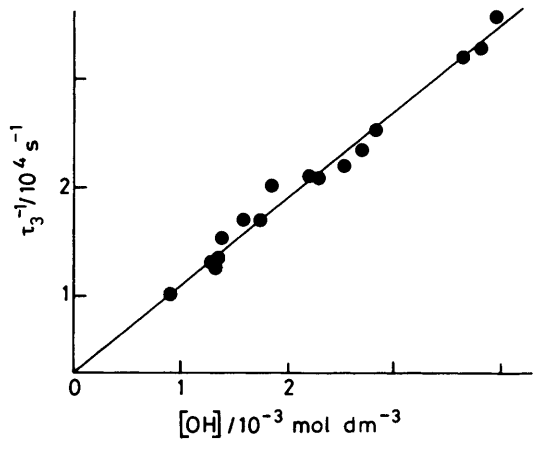


Figure 6. Plot of τ_3^{-1} *vs.* $[OH^-]$; intercept, $(2.3 \pm 1.3) \times 10^3$ s^{-1}; slope, $(7.8 \pm 1.7) \times 10^6$ dm^3 mol^{-1} s^{-1}; *r*, 0.990 01.

55% of the rate constant measured for thiamine pseudobase transformation in basic media (19.6 dm^3 mol^{-1} s^{-1}).[1] This is rather surprising, because we had assumed that the hydroxylation of the 2-position would not be affected by sulphamoylation of the amino group.[11] However, it is known from crystallographic data[10] and from the high energy of the rotation barrier of the 7'-NH_2,[11] more especially in acidic media, that thiamine probably exists as a preferred structure in which the amino group is kept close to the 2-position.[1a] Therefore, a modification of the amino group may disturb this structure, leading to a hydroxylation rate closer to those measured for thiazolium salts without the aminopyrimidine moiety.[12]

The Stopped-flow Kinetic Process.—We ascribe the fast phenomenon observed by stopped-flow to reaction (8) [formation of adduct (D')] which will rate-limit the (A') $\longrightarrow (J')^-$ transformation. The reciprocal relaxation time equation of reaction (8) is expressed as equation (13).[1c]

$$[OH^-]\tau_2^{-1} = k_{14}[OH^-]^2 + k_{41}K_{4b} \quad (13)$$

A linear least-squares regression of the data employing equation (13) gave (Figure 5), $k_{14} = (64 \pm 4)$ mol dm^{-3} s^{-1}. This value is, lower (*ca.* 60%) than that of the transformation of thiamine into its own σ-adduct (D) (99 dm^3 mol^{-1} s^{-1}).[1] The modification of the rate constant of (D') formation is expected, since the 7'-amine is probably directly responsible for reaction (8) and is more sterically hindered than that of thiamine.

The T*-Jump Kinetic Process.*—The relaxation mode observed after performing a *T*-jump on a solution of $(J')^-$ is depicted in reaction (10). The reciprocal relaxation equation associated with it can be expressed as equation (14).[1,9]

$$\tau_3^{-1} = k_{45}[OH^-] + k_{54} \quad (14)$$

A linear least-squares regression of the data employing equation (14) (Figure 6) gave, at 6 °C and an ionic strength of 0.5 mol dm^{-3}, $k_{45} = (7.8 \pm 1.7) \times 10^6$ dm^3 mol^{-1} s^{-1} and $k_{54} = (2.3 \pm 1.3) \times 10^3$ s^{-1}. The k_{45} value measured here is not very different from that of the (D) $\longrightarrow$ $(J)^-$ transformation of thiamine (5.25×10^6 dm^3 mol^{-1} s^{-1}),[1] indicating that both transformations are probably very similar.

In the case of thiamine, the transformation of (D) into $(J)^-$ was considered to involve a proton transfer occurring either on the secondary amine or at the 2-position of (D).[1]

Table 1. The rate and equilibrium constants involved in the structural transformations of thiamine and ST in basic media.

Reaction	$dm^3\ mol^{-1}\ s^{-1}$	Reverse rate constant/s^{-1}	Dissociation constant/mol dm^{-3}
$(A)^+ + OH^- \rightleftharpoons (B)$	19.2–19.6	1.15×10^{-3}	5×10^{-6}
$(A') + OH \rightleftharpoons (B')$	10.80	—	—
$(A)^+ + OH^- \rightleftharpoons (D)$	99	*ca.* 1.5	2×10^{-2}
$(A') + OH^- \rightleftharpoons (D')$	66	*ca.* 2	2.80×10^{-4}
$(D) + OH^- \rightleftharpoons (J)^-$	5.15×10^6	5.25×10^3	7.95×10^{-4}
$(D') + OH^- \rightleftharpoons (J')^-$	7.90×10^6	2.3×10^3	3.20×10^{-4}

Table 2. Chemical shifts (δ ±0.02) ppm of $(A)H^{2+}$, $(A')H^{2+}$, $(J)^-$, and $(J')^-$ and/or $(D')^-$ according to structure.

Atom	$\delta[(A)H^{2+}]$	$\delta[(A')H^{2+}]$	$\delta[(J)^-]$	$\delta[(J')^-$ and/or $(D')^-]$
C-2	155.3	148.42	153.03	31.20
C-4	143.6	144.74	139.90	142.56
C-5	136.9	135.80	129.10	127.11
4-CH_3	12.2	11.58	15.92	20.97
5-CH_2	30.3	30.53	42.26	40.83
CH_2–OH	61.4	61.05	61.3	62.02
C-2′	163.9	162.11	166.93	167.45
C-4′	163.9	162.11	166.93	167.45
C-5′	106.9	115.79	111.87	111.44
C-6′	145.5	144.74	161.73	162.91
5′-CH_2	51	49	42.80	43.84
2′-CH_3	22.10	22.10	24.16	24.62

$$(D) + OH^- \underset{k'_{54}}{\overset{k'_{45}}{\rightleftharpoons}} (D)^- \qquad (\textit{fast}) \quad (15)$$

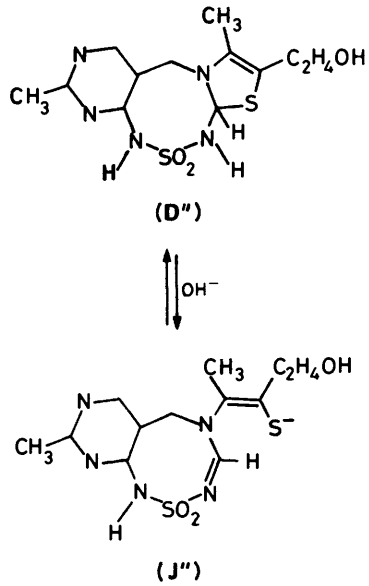
$$(D)^- \rightleftharpoons (J)^- \qquad (\textit{ultra-fast}) \quad (16)$$

We had presumed that for reactions (15) and (16) the rate-limiting step of $(J)^-$ formation from (D) would be the ring-opening of the latter.[1] However, if the deprotonation of (D) were to occur on C-2, there would be no reason to suppose that this non diffusion-controlled proton transfer, since it occurs on a carbon acid,[13] should be faster than the ring-opening of the thiazole cycle. This is chiefly because these ring-opening reactions can sometimes be diffusion-controlled.[14] In this case, the observed relaxation mode would be ascribed to reaction (15) [the deprotonation of (D)], the reciprocal relaxation-time equation associated with which is expressed as equation (17)[9] where $K' = [(J)^-]/[(D)]$.

$$\tau_3^{-1} = k'_{45}[OH^-] + k'_{54}/K' \qquad (17)$$

The k_{45} value measured for thiamine[1] may, therefore, be that for the deprotonation of (D) ($k'_{45} \approx k_{45}$). We can explain the similarity between this value and that measured for ST by assuming that both (D) and (D′) deprotonate in the same manner and at the same deprotonation site. In this case, the kinetic isotope effect reported for the (D) ⟶ $(J)^-$ transformation ($k_{45}^{OH}/k_{45}^{OD} = 2$)[1] describes directly the deprotonation of (D). The high value of this effect indicates, in the case of a base-catalysed reaction, the possible implication of a non-diffusion-controlled protolytic reaction involving a carbon acid.[13,15] All these indications seem to suggest the possible deprotonation of C-2 both in (D) and in (D′) to yield anions $(D)^-$ and $(D')^-$, respectively.

The Structural Transformation of (D′) *into* $(J')^-$.—The thermodynamics and kinetics of ST transformations in basic media resemble those of thiamine itself (Table 1). The formation of $(J')^-$ occurs in a two-step system, requiring 2 equiv. of OH^-. Although the transformation of $(A')^+$ into (D′) is slowed by one third, compared with that of $(A)^+$ into (D), it still occurs by the nucleophilic attack of an amine at the 2-position of the thiazolium ion and is still accompanied by the same proton loss at the amine which is then followed by formation of the yellow form with probably the same deprotonation step. However, the secondary amine of (D) is replaced in (D′) by a tertiary amine which prevents the formation of an analogue of $(J)^-$. Although the UV spectrum of $(J')^-$ is close to that of $(J)^-$, these two species cannot be similar unless the structure of $(J)^-$ is different from that proposed in the literature by Maier and Metzler.[8] We will not, however, consider this possibility. We can then imagine for the ST σ-adduct an 8-membered ring structure (D″), arising from intramolecular nucleophilic attack by the terminal amine of the sulphamoyl at the 2-position of $(A')^+$, which by the thiazole ring-opening is transformed into a yellow form, according to Scheme 2. It should be noted that in this

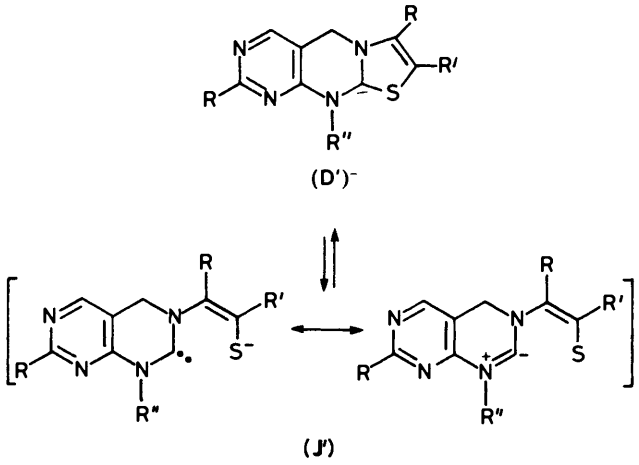


Scheme 2.

8-membered ring structure for the ST yellow form $(J'')^-$, the 2-position is identical with that of the thiamine yellow form $(J)^-$.

Although to our knowledge 8-membered rings similar to those in Scheme 2 are unknown, we cannot discard this possibility. Generally, secondary amines are considered slightly less reactive than primary ones.[16] This is true in the most common case in which tetrahedral complex formation with the amine usually takes place by attack at the nucleophilic site by the nitrogen lone pair and by the deprotonation of the ammonium formed.[16] However, in ST the secondary amine is linked to two electronegative groups (the pyrimidine ring and SO_2NH_2) which should considerably decrease the pK value for proton loss.[13] In basic media, this amine can be in its anionic form and is, therefore, probably more reactive than the terminal NH_2 of the sulphamoyl group. It would, then, yield by reaction (8) the 6-membered σ-adduct (D′) (Scheme 1). This possibility was investigated by ^{13}C NMR spectroscopy.

The Structure of the ST Yellow Form and the Role of the Aminopyrimidinyl.—The $(J)^-$ and ST yellow form are kinetic products with short lifetimes which render their structural analyses in purely aqueous media difficult and time-limited.[1,17] However, species $(J)^-$ can be stabilized in basic methanol for several hours[8a] at concentrations which, nevertheless, are



Scheme 3.

inadequate for ^{13}C NMR spectroscopy. The ^{13}C NMR spectra of $(J)^-$ and the ST yellow form were, therefore, acquired in an aqueous alcoholic solution at low temperature (−10 °C), in which the life span of these two species is 3–4 h.

The ^{13}C NMR spectrum of $(A')H^{2+}$ displays 10 carbon signals, one of which (49.0 ppm) is assigned to the resonance of the methanol carbon.[18] We assign the other signals to the various carbon atoms by direct comparison with the published spectrum of $(A)H^{2+}$ (Table 2).[19] The spectrum of $(J)^-$ displays 11 carbon signals which we assign to the 12 carbon atoms of the molecule, according to published data and by comparison with the spectrum of $(A)H^{2+}$ (Table 2). In the spectrum of $(J)^-$ the signals at 153.03, 161.73, and 166.93 ppm are typical of carbon atoms in pyrimidines.[18,19] The C-2 and C-6′ signals (if recorded without proton decoupling) should appear as two doublets with C–H coupling constants in the 170–210 Hz range.[20] However, the lifetime of the $(J)^-$ species and its solubility in the alcoholic medium do not allow the acquisition of the ^{13}C spectrum without proton decoupling. We used, therefore, the distortionless enhancement by polarization technique (DEPT)[21] in order to acquire the signals of the carbon atoms of $(J)^-$ which only bear 1-H by estimating the average coupling constant of the pyrimidine C–Hs at *ca.* 190 Hz.[20] This spectrum shows that the signals at 161.73 and 153.03 ppm are those of carbon atoms attached to one proton. We therefore assign the signals at 161.73 and 153.03 ppm to C-6′ and C-2, respectively, and that at 166.93 to C-2′ and C-4′ (the 2-position in the pyrimidine ring)[18,19] as for $(A)H^{2+}$ for the signal at 163.9 ppm.[19] All other signals can be easily assigned to the other carbon atoms. All upfield and downfield shifts from the signals of $(A)H^{2+}$ are explained (i) by the disappearance of the positive charge on 1-N and the ring-opening of the thiazole for C-4, 5′-CH_2, and 4-CH_3 and (ii) by the ring-opening of the thiazole and the appearance of a negative charge at the sulphur atom for C-5 and 5-CH_2, and by the formation of a new 6-membered ring for C-5′.[18] The spectrum of the ST yellow form recorded under the same experimental conditions shows at least one major difference between that of $(J)^-$ (Table 2): the C-2 signal at 153 ppm is shifted upfield either to 40.83 or to 31.20 ppm. The DEPT spectrum, performed in order to acquire the signals of carbons bearing several protons[21] by estimation of the average coupling constant of the CH_2s of the ST yellow form at 140 Hz,[18,20] shows that the signals at 40.83, 43.84, and 62.02 ppm are those of carbons bearing two protons. We can, therefore, assume that the C-2 in the ST yellow form resonates at 31.20 ppm which cannot be the case for C-2 in the 8-membered $(J'')^-$.[18,19] Furthermore, the signal at 31.20 ppm cannot be ascribed to C-2 in 6-membered (D′) or in 8-membered (D″), which are both linked to 1-S and 2-N and should, therefore, resonate at lower fields.[18] The very important upfield shift (118 ppm) between the C-2 signals in $(J)^-$ and the ST yellow form cannot be easily interpreted. These shifts are reported when the charge density on the carbon atom is inverted, *i.e.* there is a difference of 118 ppm upfield between the signals of the α-carbon of the triphenylmethyl cation and anion.[22] Moreover, the appearance of a negative charge on a carbon atom is usually accompanied by upfield shifts which are much lower than the 118 ppm observed between the C-2s of $(J)^-$ and that of the ST yellow form.[22] However, a negative charge invevitably induces changes in the bonding situation of the carbon. This can generate shift changes other than those expected from the modification in the charge density of the atom.[22b] As shown by the kinetic analysis, the structural transformations of ST are similar to those of thiamine with, however, a 2-position in $(J')^-$ different from that of $(J)^-$. $(A')^+$ Can yield a 6-membered ring (D′) which can deprotonate in $(D')^-$ according to Scheme 1 and its ring-opening (if it occurs) would, therefore, take place according to Scheme 3. In this case, the 31.20 ppm signal can be ascribed to the negative C-2 in (J′) and/or in $(D')^-$ (Table 2).

We are here cautiously speculating in implying that the structure of the ST yellow form may be that of $(D')^-$ which, in the case of ring-opening, yields a resonance-stabilized carbene species. $(J')^-$ can be compared to Breslow's $(A)_{-H}$[4] which is believed to be responsible for the metabolic activity of vitamin B_1.[2,3,8,19,23] The fact that the C-2 can deprotonate during the $(D') \longrightarrow (J')^-$ transformation does not exclude the direct protonation of this C-2 in $(A')^+$ to yield a resonance stabilized carbene species $(A')_{-H}$ similar to $(A)_{-H}$. However, the direct deprotonation of C-2 in thiamine has an estimated pK value of 18 and is considered to be diffusion-controlled,[5] whereas the formation of $(J')^-$ from (D′) occurs with pK_4 = 10.50 and the proton transfer involved is not diffusion controlled.[1] We can, therefore, as in the case of thiamine,[1] exclude the involvement of the $(A')_{-H}$ species in the $(A')^+ \longrightarrow (J')^-$ transformation.

Although, as indicated recently, the aminopyriminyl group does not provide significant intramolecular catalysis in the formation of $(A)_{-H}$,[5] it is necessary for the enzymic and non-enzymic catalytic activity of the vitamin,[3,4,19,23] and the absence of 7′-NH_2 is known to inhibit this catalytic activity.[23] Furthermore, even if the replacement of one of the protons of this amino group by a sulphamoyl group modifies the rates and equilibrium constants of the reactions involved in the general mechanism of the structural transformations, these reactions still occur in basically the same way, as evidenced by kinetic analysis. Therefore, if the catalytic activity of thiamine does not only stem from $(A)_{-H}$ but also from $(D)^-$, the replacement of one of the two protons of the 7′-NH_2 by a small alkyl group such as methyl should not markedly affect the deprotonation of adduct (D) into $(D)^-$, nor should it affect the catalytic efficiency of the transformed thiamine. Breslow showed that, in such a transformed thiamine, the catalytic efficiency in benzoin condensation is only decreased by *ca.* 30%.[4] This provides further support for the proposal of $(D)^-$ as a biocatalyst for the metabolic reactions involving vitamin B_1.[1]

Conclusions

The mechanism for the structural transformation of ST in basic media resemble those of thiamine itself. $(A')^+$ is transformed with one OH^- molecule into adduct (D′) which is in turn transformed with a further OH^- equiv. into a yellow form. However, (D′) cannot undergo a deprotonation at the tertiary amine and cannot yield a yellow form analogous to that of $(J)^-$. Therefore, the most reasonable reversible transformation involving a proton loss that (D′) can undergo is the

deprotonation of the 2-site and perhaps the ring opening of the $(D')^-$ anion into a resonance-stabilized carbene species resembling Breslow's catalyst $(A)_{-H}$. Moreover, the occurrence of the proton loss during the $(D') \longrightarrow (J')^-$ transformation, with a rate similar to that of $(A)^+ \longrightarrow (J)^-$ seems to confirm the existence of anion $(D)^-$ which we regard, together with $(A)_{-H}$, to be a promoter of the biocatalytic activity of vitamin B_1.

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