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Model Studies of the Thymidylate Synthase Reaction. The Mechanism of Reduction of 5-Uracilylmethylenepyridinium Salts by Benzyl Thiol

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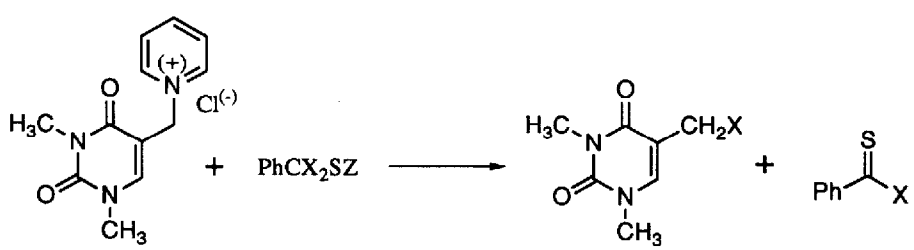
Abstract : The reduction of 5-uracilylmethylenepyridinium salts by benzyl thiols, to the corresponding thymine derivatives, involves an exocyclic methylene intermediate, which appears to be reduced by a radical mechanism. The overall reaction constitutes a mechanistic model of the reduction step of the thymidylate synthase reaction.

In connection with our studies of model reactions of the thymidylate synthase (TS) catalyzed transformation of dUMP to dTMP[2], we have investigated the reaction of 5-uracilylmethylenepyridinium salt (**1**) with thiols, as a mimic of the reduction step. In a previous communication[3] we had suggested that the reduction of **1** to thymine derivative **3a**, by benzyl thiol **2a**, involved the transfer of the thiol RSH hydrogen, as a hydride equivalent. (Scheme 1). We now present results which identify the source of the transferred-hydrogen and throw light upon the mechanism of transformation of **1** to **3a**.



1

2a X = Z = H
2b X = H, Z = D
2c X = D, Z = H

3a X = H
3b X = D

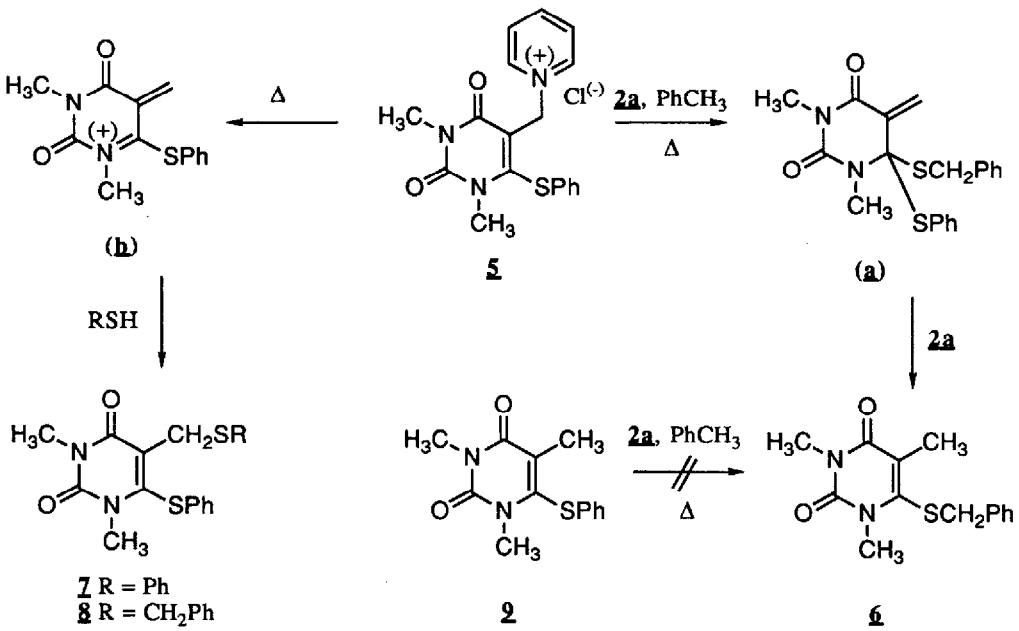
4

Scheme 1

The quest for the source of the hydride equivalent led us to synthesize both, $PhCH_2SD$[4] (**2b**) and $PhCD_2SH$[5] (**2c**) and employ these labelled thiols for the reduction reaction. The reaction of **1** with $PhCH_2SD$ (**2b**) led to product **3a**, in which no deuterium was incorporated. On the other hand, when **1** (0.3 mmol) and **2c** (0.75mmol) were refluxed in toluene (2 days), the thymine derivative **3b**, containing one

deuterium atom, was formed in 95% yield. The position and the extent of the label in **3b** was attested by its NMR and mass spectra[6]. These results show unequivocally that during the reduction process, a hydrogen from the α-position of the benzyl thiol is transferred to an intermediate generated during the reaction. The formation of the oxidation product of benyl thiol, namely, thiobenzaldehyde (**4**), was demonstrated (in a reaction with unlabelled thiol) by the isolation and identification of its cyclic aminal derivative with $PhNHCH_2CH_2NHPh$[7].

Having identified the source of the reducing equivalent, we addressed our attention to the nature of the intermediate or intermediates involved in the reaction. An insight into this was obtained by the study of the reaction of salt **5**[8] with thiol **2a** (Scheme 2). The products of this reaction were **6** (35%), **7** (32%) and **8** (28%), all of which were identified by their NMR spectra[9].

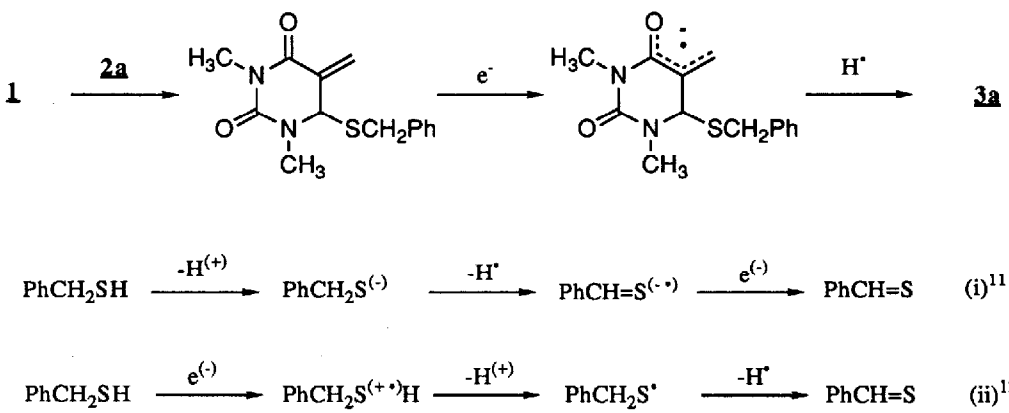


Scheme 2

The formation of **6** suggests that it arises from an intermediate in which the thiol **2a**, at some stage, had added to the C(6)-position of the uracil moiety of **5**. A plausible candidate for this intermediate is the exocyclic methylene thioacetal (**a**). The observed product **6** is formed by reduction of (**a**), by transfer of a hydride equivalent from **2a**, followed by selective expulsion of the better leaving phenyl mercaptide ion. That the thiol residue-exchange did not occur subsequent to the reduction step, was demonstrated by the fact that, under conditions of the reaction, thymine **9** is unchanged upon reaction with **2a**. Since a nucleophilic attack on the methylene group of intermedate (**a**) is expected to expel the phenyl mercaptide ion, the formation of thioethers **7** and **8**, in which the C(6)-SPh substituent is retained, can be best rationalized on the basis of a second pathway involving intermediate (**b**). The generation of this intermediate can be visualized via a thermally induced fragmentation of salt **5**. The quenching of intermediate (**b**) by the mecaptide ion of **2a** ($PhCH_2S^-$), present in

the reaction mixture, or by PhS^-, released concomitantly upon reduction of (**a**), accounts for the formation of **7** and **8**.

In connection with the nature of the reducing equivalent, [that is, hydride versus electron + hydrogen radical; SET mechanism], it is particularly noteworthy that the reaction of **5** with **2a** does not lead to a thymine derivative in which the C(6)-SPh substituent is retained. This would suggest that an intermediate such as (**b**) is not reduced under the conditions of the reaction. Since reduction of such iminium ions by hydride reducing agents has been shown in other experiments[10], one may question the ability of benzyl thiol to reduce via the delivery of a formal hydride species. In view of the aforementioned we would like to suggest an alternate mechanism involving an e + $H^\bullet$ for the reduction of salt **1** by benzyl thiol (**2a**) (Scheme 3). Support for the latter is derived from a galvinoxyl assay of the reaction. When the influence of galvinoxyl radicals on the reaction of **1** with **2a**, was monitored, the rate of formation of **3a** (t 1/2) progressively decreased with increasing concentration (eq.) of the scavenger radicals [t 1/2 = 15 hr (0.0 eq.), 60 hr (1.0 eq.), >150 hr (2 eq.)].



Scheme 3

It is interesting to speculate on the mechanistic details of the oxidation of $PhCH_2SH$ (**2a**) to PhCHS. Two plausible mechanisms are described in pathways (i)[11] and (ii)[12], which differ in the sequence of transfer of the e^-, H^+ and $H^\bullet$ species. Studies are in progress which are expected to throw light on the details of the mechanism of oxidation of **2a** during the reaction.

The overall mechanism for the reduction of pyridinium salt **1** by **2a** bears analogy to the reduction step of the thymidylate synthase reaction in which an enzyme-bound exocyclic methylene intermediate is reduced via a radical mechanism by the coenzyme tetrahydrofolate[13].

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REFERENCES AND NOTES

* To whom correspondence should be addressed.

1. Taken in part from the forthcoming doctorate dissertation of Drs.J.W.G.Meissner, University of Amsterdam.
2. van der Meij, P.F.C.; Chen, T.B.R.A.; Hilhorst, E.; de Waard, E.R.; Pandit, U.K. *Tetrahedron*, **1987**, *43*, 4015.
3. Vega, E.; Rood, G.A.;de Waard, E.R.; Pandit, U.K. *Tetrahedron*, **1991**, *47*, 4361.
4. $PhCH_2SD$ was prepared by H/D exchange with D_2O.
5. $PhCD_2SH$ was prepared from PhCOOMe via the sequence: i $LiAlD_4$; ii Ph_3P/CCl_4; iii $S=C(NH_2)_2$; iv NaOH.
6. ^1H NMR (200 MHz; $CDCL_3$): 1.94 (m, 2 H, $C_5C\underline{H}_2D$), 3.36 (s, 3 H, $NC\underline{H}_3$), 3.37 (s, 3 H, $NC\underline{H}_3$), 6.99 (s, 1 H, H_6). Accurate mass 155.0812 (Calcd for $C_7H_9N_2O_2D$ 155.0805).
7. The cyclic aminal was identified by comparison (NMR, TLC) with an authentic sample prepared by the reaction of the diamine with benzaldehyde.
8. The preparation of this salt will be described in a forthcoming detailed publication.
9. Compound **6** ^1H NMR (200 MHz; $CDCl_3$): 2.09 (s, 3 H, $C_5C\underline{H}_3$), 3.35 (s, 3 H, $NC\underline{H}_3$), 3.49 (s, 3 H, $NC\underline{H}_3$), 3.94 (s, 2 H, $SC\underline{H}_2Ph$), 7.10 - 7.45 (m, 5 H, $SCH_2\underline{Ph}$).
   Compound **7** ^1H NMR (200 MHz; $CDCl_3$): 3.35 (s, 3 H, $NC\underline{H}_3$), 3.39 (s, 3 H, $NC\underline{H}_3$), 3.87 (s, 2 H, $C_5C\underline{H}_2$), 7.10 - 7.45 (m, 10 H, 2 x $S\underline{Ph}$).
   Compound **8** ^1H NMR (200 MHz; $CDCl_3$): 3.39 (s, 3 H, $NC\underline{H}_3$), 3.54 (s, 3 H, $NC\underline{H}_3$), 3.97 (s, 2 H, $C\underline{H}_2$),4.08 (s, 2 H, $C\underline{H}_2$), 7.10 - 7.45 (m, 10 H, $S\underline{Ph}$ and $SCH_2\underline{Ph}$).
10. Salt **1** can be reduced to thymine **3a** by reaction with Hantzsch ester or 9,10-dihydrophenanthridine. See reference 2.
11. Casarini, D.; Lunazzi, L.; Placucci, G. *J. Org. Chem.*, **1988**, *53*, 1582.
12. We wish to thank a referee for drawing our attention to this pathway.
13. (a) Slieker, L.J.; Benkovic, S.J. *J .Am. Chem. Soc.*, **1984**, *106*, 1833.
    (b) Santi, D.V.; Danenberg, P.V. in *Folates and pterines*, Vol. 1 (Blakely, R.L.; Benkovic, S.J.; eds), Wiley, New York, **1984**, 345 - 398.

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