A Chemical Model of Thymidylate Synthetase. Formation of a Thymine Derivative *via* an Exocylic Methylene Intermediate†

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Reaction of 6-aminouracil derivatives with imidazolidines (methylenetetrahydrofolate models), in the presence of acid, gives an exocyclic methylene intermediate which reacts with nucleophiles present in the mixture or can be reduced to a thymine derivative.

The thymidylate synthetase catalysed conversion of dUMP to dTMP involves the overall transfer of a 'CH_3' group from the coenzyme 5,10-methylenetetrahydrofolate to the substrate, deoxyuridine monophosphate. The currently accepted mechanism of action of the enzyme[1] visualizes a carbon transfer step, leading to formation of an exocylic methylene intermediate, followed by reduction of the latter to the thymine derivative (dTMP). In this communication we present a chemical model of thymidylate synthetase that involves the formation of an exocyclic methylene intermediate which can be reduced to a thymine derivative.

Studies in our laboratory have demonstrated that unsymmetrically substituted imidazolidines can serve as models of the coenzyme 5,10-(substituted)methylenetetrahydrofolate in mediating carbon transfer reactions.[2] Furthermore, it has been suggested by us that 6-aminouracil derivatives may be regarded as mimics of the apoenzyme–substrate complex of the thymidylate synthetase catalysed reaction.[3] Combining these concepts, it follows that the reaction of 6-aminouracils with imidazolidines should serve as a model for the enzymatic process. The reaction between (**1a**) and (**2a**), leading to (**3a**), was assumed to follow the 'enzyme-like' pathway [involving the exocyclic methylene intermediate (A), R = Me] for the carbon transfer step.[3] We now present evidence for the formation of intermediate (A) and show that it can be reductively quenched to the corresponding thymine derivative.

In the reaction of (**1a**) with (**2b**) (1 : 1) in CF_3CO_2H–MeCN (1 : 3) at room temperature, after 2 h the imidazolidine (**2b**) has completely reacted and the reaction mixture consists of (**1a**), (**4b**), and (**5a**) in the ratio 1 : 1 : 1 and a small amount (~ 5%) of (**6a**) (h.p.l.c.).‡ After a longer reaction time,

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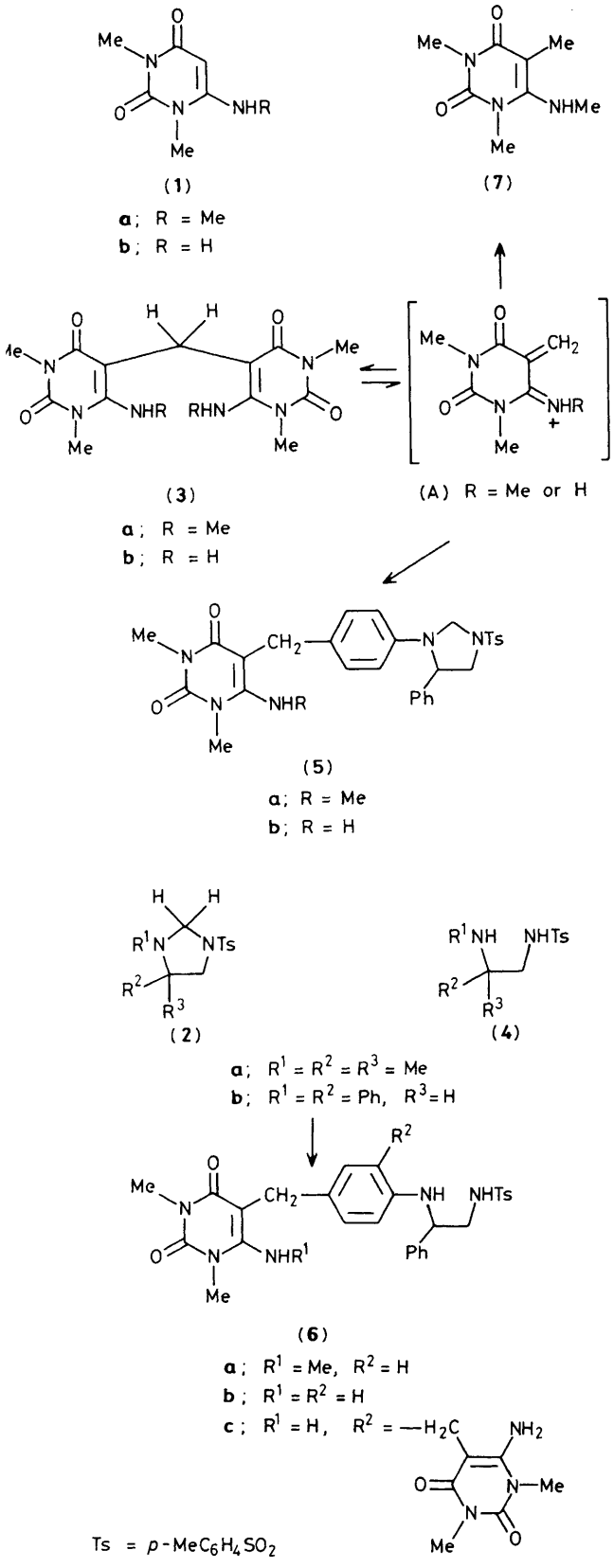
‡ H.p.l.c. column: PE/HS-5 C18, 125 × 4.6 mm; mobile phase: MeCN–H_2O, 55 : 45 (v/v); flow rate: 1 ml/min.



h.p.l.c. analysis shows that (**6a**) increases at the expense of the products (**1a**), (**4b**), and (**5a**) and after 24 h constitutes about 95% of the reaction mixture. Reaction between (**1b**) and (**2b**) under analogous conditions occurs rapidly and monitoring of the mixture by h.p.l.c. exhibits the following changes. After 30 s no (**1b**) is present; the mixture consists of (**2b**), (**3b**), and (**4b**) (1 : 1 : 1). After 10 min (**2b**) has disappeared and (**3b**), (**4b**), and (**5b**) (*ca.* 1 : 2 : 1) appear as the main products. Finally, after 24 h, the mixture contains (**6b**) as the major (> 80%) constituent.§ All these results can be rationalized in terms of the generation and reactions of intermediate (A).[3] Initial formation of (**5a**) is accounted for by the reaction of (A) with (**2b**) in an electrophilic substitution process on the anilino moiety of (**2b**). Compound (**5a**), in turn, slowly transfers a methylene group to (**1a**) and the resulting (additional) intermediate (A) reacts with (**4b**) to yield the uracil derivative (**6a**). It is implicit in this rationalization that reaction of (A) with (**2b**) is faster than with (**4b**). The rapid formation of (**3b**), in the reaction of (**1b**) with (**2b**), again attests to the generation of (A) and its subsequent reaction with (**1b**) in a kinetically controlled step. Since (**3b**) disappears with the resulting formation of (**6b**), it would appear that under the reaction conditions the formation of (**3b**) is reversible and that (A) reacts with (**4b**) to give the thermodynamically stable product (**6b**). This explanation is supported by the reaction of a 1 : 1 mixture of (**3b**) and (**2b**) in CF_3CO_2H–MeCN (1 : 3) at room temperature. The main product is (**6c**), which is consistent with the sequence: (i) breakdown of (**3b**) into (**1b**) and (A), (ii) reaction of (A) with (**2b**) to give (**5b**), (iii) methylene group transfer from (**5b**) to (**1b**), resulting in (A) and (**6b**), and finally, (iv) electrophilic reaction of (A) with (**6b**), at the *ortho*-position of the anilino group.

It has been possible to show that the formation of (**3a**), under acidic conditions, is reversible with respect to (**1a**) and intermediate (A). Thus, when an authentic sample of (**3a**), in acetic acid, was hydrogenated over Pd/C (10%), the substrate was quantitatively converted into a mixture of uracil (**1a**) and thymine derivative (**7**). The latter product is obviously formed by the reductive quenching of the exocyclic methylene intermediate (A). Experiments designed to mimic the enzymatic reduction of (A) are currently in progress.

The structure of all new compounds has been established by satisfactory analytical and/or spectral data.

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§ The chromatogram shows minor amounts of (**4a**) and the disubstituted product (**6c**).