CHEMICAL PRECEDENCE FOR REDUCTION OF THE "EXOCYCLIC METHYLENE" INTERMEDIATE IN A TETRAHYDROQUINOXALYL MODEL OF THYMIDYLATE (TMP) SYNTHETASE.

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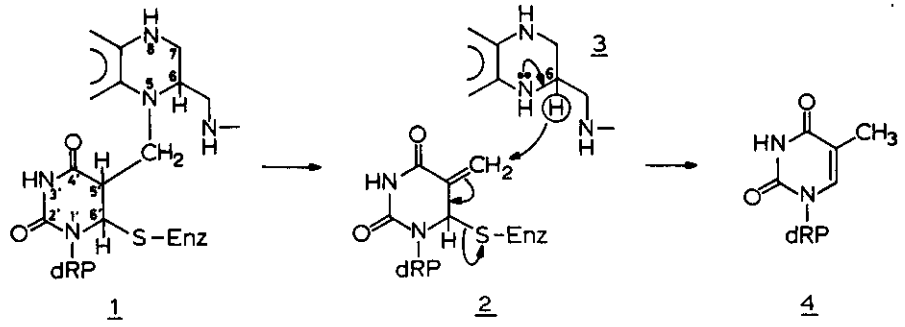
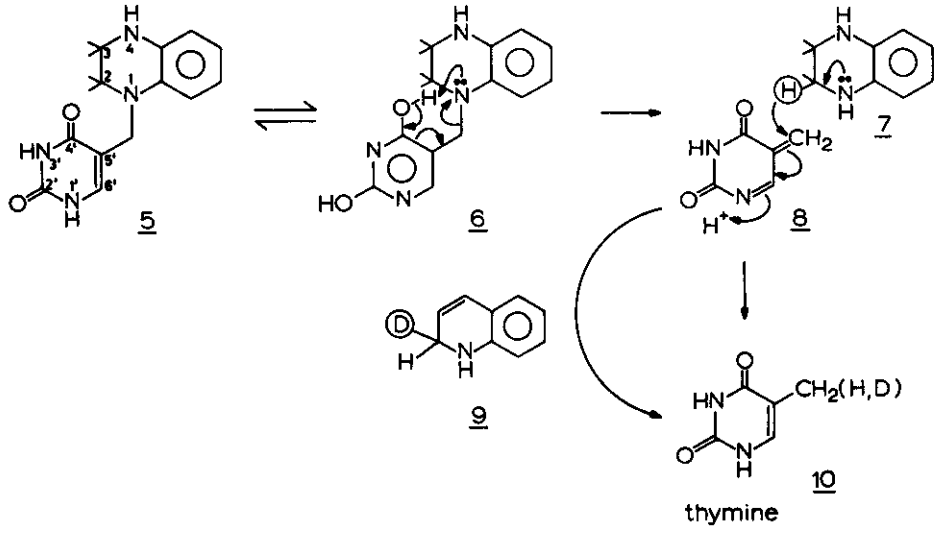
Abstract - 1,2,3,4-Tetrahydro-1-(thyminyl)quinoxaline undergoes thermal conversion to thymine and tetrahydroquinoxaline. The formation of thymine has been shown to involve an intermolecular hydrogen transfer to an exocyclic methylene intermediate analogous the one proposed in the TMP synthetase reaction.

Direct evidence for the formation of a ternary complex in which both the cofactor 5,10-methylenetetrahydrofolate and the substrate analogue FdUMP (5-fluorodeoxyuridine-5'-phosphate) are tightly bound to the enzyme[2], has lent support to the suggestion that the catalytic mechanism is initiated by attack of a cysteinyl sulphydryl group[3a-f] located at the active site, on C(6') of the pyrimidine ring, to generate a carbanion at C(5'), which subsequently attacks the 5,10-methylenetetrahydrofolate to give intermediate 1 (Scheme I)[4a,b]. The latter intermediate can, in principle, be converted into the exocyclic methylene species 2 and tetrahydrofolate 3 via two pathways: (a) Protonation of N(5), followed by loss of C(5')-H, with concomitant elimination of tetrahydrofolate (3), and (b) enolization of the C(5')H - C(4')=O group in 1 and fragmentation of the resulting enol through a six-membered ring transition-state, to a mixture of 2 and 3. Completion of the process, that is formation of TMP (4), would occur by transfer of the hydrogen from 3 to 2 (Scheme I) and dissociation of the enzyme complex[4a,b]. In this communication we present results which provide chemical precedence for both, the fragmentation of 1 via pathway (b) and the intermolecular hydrogen transfer to an intermediate analogous to 2. 1,2,3,4-Tetrahydro-1-(thyminyl)quinoxaline (5, Scheme II) was chosen as a model for intermediate 1. Although the corresponding quinoline derivative has been studied earlier[5a,b], it was felt that the reduced quinoxaline system bears a closer analogy to the tetrahydropteridine moiety of the folate cofactor. Furthermore, despite the fact that the pyrimidinyl C(5') in 5 is in the sp^2-hybridized state and does not carry a hydrogen atom, as in 1, it was envisaged that the sys-



Scheme I



Scheme II

tem could fragment via its tautomeric form 6 into tetrahydroquinoxaline 7 and the "exocyclic methylene" equivalent 8, which would be highly susceptible to reduction to thymine. The model 5 was conveniently obtained by coupling quinoxaline with 5-chloromethyluracil, followed by reduction of the quinoxalinium salt with sodium borohydride (5, m.p. 230°d; NMR DMSO-d_6 δ 3.32 - 3.45 m, 4H, quinoxaline $-CH_2CH_2-$; 4.00 broad s, 2H $-CH_2-$; 6.42 - 6.63 m, 4H Ar-protons; 7.18 d, 1H C(6')-H, J_{H_1,H_6} = 2.5 Hz). The model compound 5 was heated (neat, 225°) under reduced pressure (.003 mm) and the volatile components separated and analyzed. These consisted of tetrahydroquinoxaline (7, 62 p.c.) and thymine (10, 8 p.c.). A fraction of the starting material was converted into decomposition products, which remained as a residue. The formation of 7 and 10 can be rationalized on the basis of the mechanism described in Scheme II. Following the anticipated fragmentation of 5, the tetrahydroquinoxaline (7) reduces intermediate (8). The discrepancy between the amounts of 7 and 10 observed, can be understood, since intermediate 8 would be readily diverted to condensation side-products and furthermore, the reductant 7 is rapidly removed from the mixture, owing to its volatility under the reaction-conditions. The mechanism of the hydrogen-transfer step described in Scheme II was supported by the fact that a five-fold enhancement (lower limit) in the yield of thymine was obtained in an experiment in which an equivalent of 1,2-dihydroquinoline[6] - a better hydride donor than 7 - was added to 5, in the reaction. An intermolecular hydride transfer was established by employing 1,2-dihydroquinoline-2-d_1 (9)[7], whereupon the thymine formed was found to be labelled (27 p.c. D) in the methyl group. In accordance with the mechanism of fragmentation of 6, visualized in Scheme II, 1,2,3,4-tetrahydro-1-(thyminyl)quinoxaline-2,3-d_2 yielded monodeuteromethylthymine[8] under the aforementioned conditions, while no deuterium label in the methyl group of the thymine[8] was observed when N_1-, N_3-, N_4-trideuterated derivative 5 was subjected to the same reaction. Also consistent with the mechanism was the lack of formation of dimethylthymine, when N_1-, N_3-dimethyl-(5), which cannot undergo the tautomerization $5 \rightleftharpoons 6$, was heated in an analogous manner. These results provide compelling evidence for the generation and intermolecular reduction of the methylene pyrimidinyl derivative 8, in the formation of thymine from a system related to the enzyme-bound intermediate 1. The results also emphasize and elucidate the important role of the C(5')-pyrimidinyl proton in the enzyme-cofactor-substrate complex (1), in the TMP-synthetase reaction; it being recalled that replacement of this proton by a fluorine atom leads to inhibition of the enzyme.

It is recognized by us that the reaction conditions (neat 225°) necessary for the fragmentation of 5, into the exocyclic intermediate (8) and 7, are very much more drastic than those under which the enzyme-catalyzed reaction proceeds in the living cells. Studies towards the development of models illustrating the formation and fragmentation of systems corresponding to 1. under mild conditions, are currently in progress.

1. Taken in part from the forthcoming doctorate dissertation of R. Plemp.
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7. Quinoline-2-d_1, obtained by decarboxylation of quinaldinic acid labelled at the carboxylic acid hydrogen, was reduced to 9, according to the procedure described in ref. 6.
8. The presence or absence of deuterium label in the methyl group of thymine was established by detailed mass spectral analysis.

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