Pure & Appl. Chem., Vol. 66, No. 4, pp. 759–764
Printed in Great Britain.

Models of biological systems and biological processes

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Abstract - Models of folate cofactors have been designed for the biomimetic transfer of a C_1-unit at the oxidation levels of formate, formaldehyde and methanol. Suitable models of 5,10-methylenetetrahydrofolate and 5-methyltetrahydrofolate have been employed for investigating mechanistic aspects of the thymidylate synthase and methionine synthase reactions. The functional capability of carbon-transfer of the models has been applied to the evolution of a strategy for practical synthesis.

The molecule pteroylglutamate (**1**, Fig. 1) constitutes the structural template of several cofactors which are involved in one carbon metabolism. The C_1-unit derivatives of its tetrahydro form (**2**), as the corresponding polyglutamates, mediate the biological transfer of carbon-fragments at the oxidation levels of formate, formaldehyde and methanol (ref. 1). The carbon to be metabolized is attached to N(5), N(10) or both N(5) and N(10) of the tetrahydrofolate system.

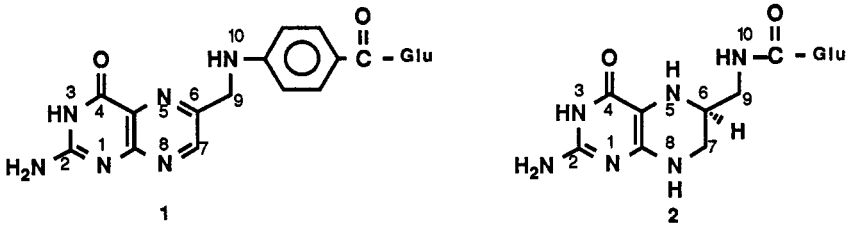


Fig. 1

Our laboratory has been engaged in designing models of the folate cofactors and investigating their chemistry with two parallel objectives (ref. 2). These being, on one hand, achieving a better understanding of molecular mechanisms of the enzymic processes in which these cofactors play an essential role, and, on the other, the development of strategies for chemical synthesis based on the reactivity patterns of the cofactors. In the latter context, it should be emphasized that many of the organic cofactors, of which the folates are illustrative, may be regarded as nature's chemical reagents (ref. 3). These cofactors are consumed, during the biochemical transformation, and have to be regenerated by one or more enzyme-

catalyzed reactions, for the mediation of the subsequent cycle. This presentation will describe some salient aspects of the studies with models which mimic three folate cofactors, namely, 5,10-methenyl-tetrahydrofolate (**3**), 5,10-methylenetetrahydrofolate (**4**) and 5-methyltetrahydrofolate (**5**), (Fig. 2). It may be noted that these tetrahydrofolate cofactors incorporate the transferable carbon unit at the oxidation levels of formate, formaldehyde and methanol.

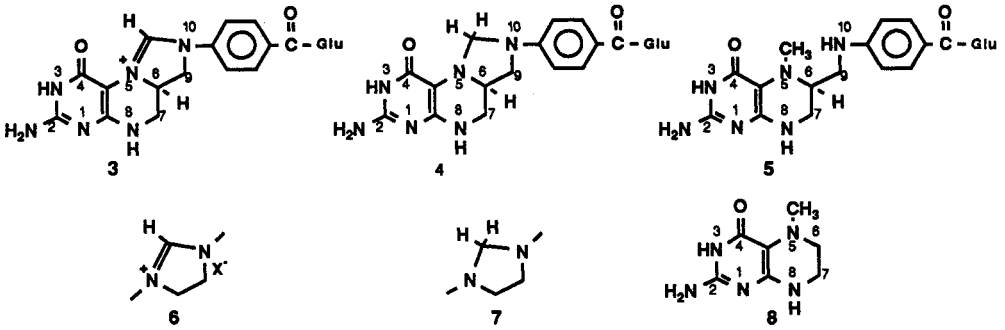


Fig. 2

Models of general structures **6**, **7** and **8** have been designed by us to simulate the functions of the cofactors **3**, **4**, and **5**, respectively (Fig. 2). These systems can be prepared conveniently from readily available starting materials and may be employed as shelf reagents (ref. 4); two of them being commercially available at present (ref. 5). In designing models **6** and **7**, the simulation of the reduced imidazole systems of the cofactors, with its unsymmetrically substituted nitrogens, was considered essential for the role of the models as potential C_1-unit transfer reagents. In case of model **8**, the functional significance of the pterin moiety in cofactor **5** is emphasized.

5,10-Methenyltetrahydrofolate Model Mediated Carbon Transfer.

The reactions of imidazolinium salt **9**, described in Fig. 3, are illustrative of the functional potential of the 5,10-methenyltetrahydrofolate models (ref. 6). Both monofunctional and bifunctional nucleophiles extrude the formate carbon from the model to result in the formation of the corresponding *transfer-products* in high yields. The ability of the models in mediating efficient carbon-transfer at the formate level, is convincingly established by these examples. It will be recognized that the cofactor itself and its models of type **6**, constitute activated formic acid derivatives and as such they extend the arsenal of existing reagents for the introduction of a formate carbon.

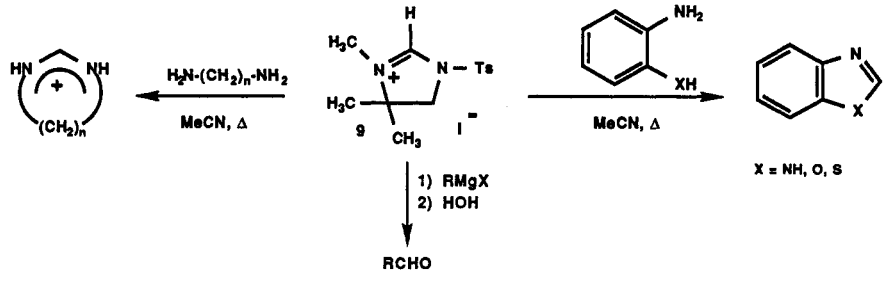


Fig. 3

5,10-Methylenetetrahydrofolate Model Mediated Carbon Transfer.

The model **10**, under influence of acid, readily transfers its methylene group, to suitable nucleophiles (ref. 7). Two typical examples are presented in Fig.4. The transfer to uracil derivatives of type **11** was specifically designed to mimic the carbon-transfer step of the thymidylate synthase (TS) catalyzed transformation of dUMP to dTMP (Fig. 5). It should be recalled that the enzyme (TS) requires 5,10-methylenetetrahydrofolate (**4**) as an essential cofactor (ref. 8). Especially noteworthy in this connection is the role of the cofactor as a reagent for the transfer of both a methylene group and a hydride equivalent.

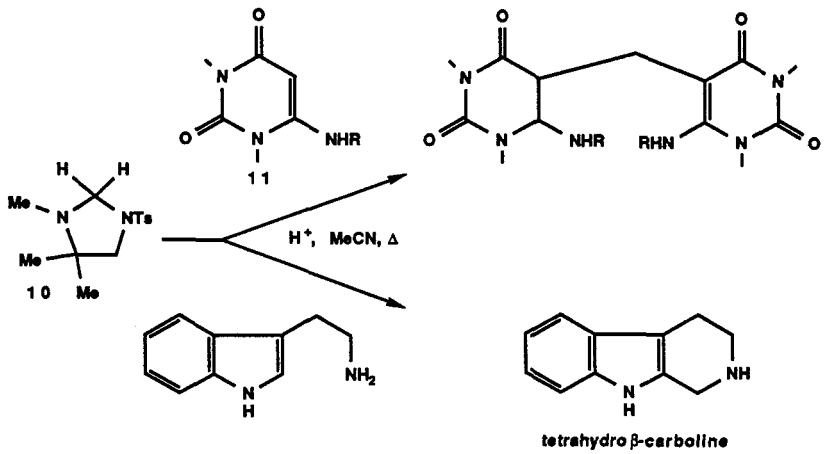


Fig. 4

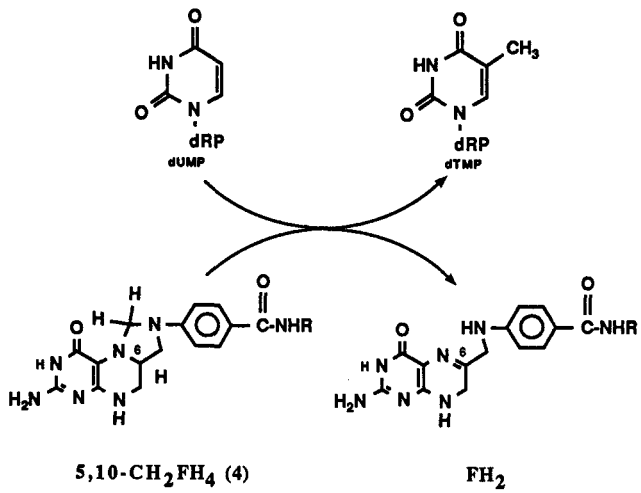


Fig. 5

A detailed investigation of the reaction of model **12** with 6-aminouracil derivatives (exemplified by **13**) revealed that an exocyclic methylene intermediate (**14**, Fig. 6), is involved during the methylene transfer process (ref. 9). This intermediate has been identified by the formation and isolation of its adduct, with 1-benzyl-3-carbamoyl-1,2,3,4-tetrahydroquinoline, whose structure has been unambiguously established by X-Ray crystallographic analysis (ref. 10). Intermediate **14** bears resemblance to the exocyclic methylene intermediate **15** postulated in the mechanism of the TS reaction.

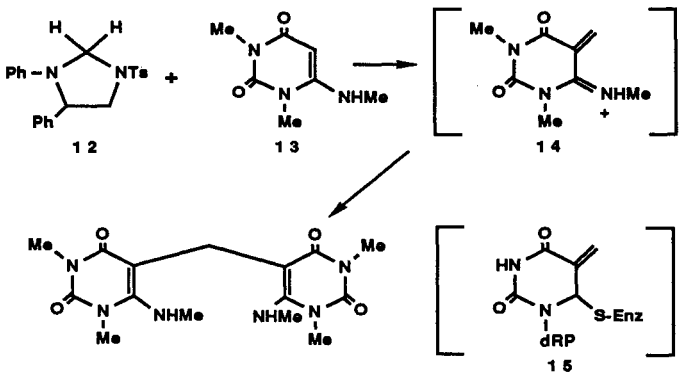


Fig. 6

In a further study directed to the simulation of the overall methyl transfer during the TS reaction, the 5,10-methylenetetrahydrofolate model **16** (Fig. 7) was developed (ref. 11). Reaction of **16** with uracil derivative **13**, in the presence of acid, showed that the model was indeed competent in transferring both a methylene moiety and a hydride equivalent to the substrate. This reaction constitutes the first non-enzymatic precedent of the conversion of a uracil derivative to the corresponding thymine system by a folate cofactor model.

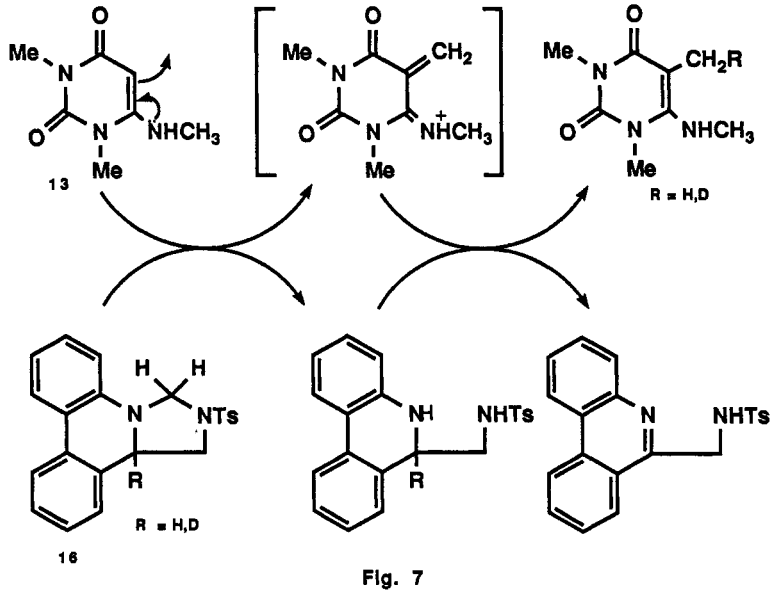


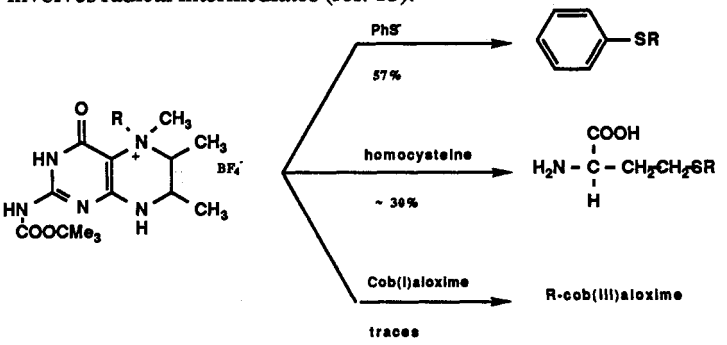
Fig. 7

In comparing the two intermediates **14** and **15** (Fig. 6), admittedly, there is a difference between their oxidation states. The intermediate of the non-enzymatic process (**14**) being at a higher oxidation level (at C-6) than the corresponding intermediate of the enzymatic reaction (**15**). Recently, it has been possible to generate a model of the (enzymatic) allylic thioether intermediate **15**. Preliminary studies reveal that such an intermediate is prone to reduction by a thiol, presumably via a radical mechanism (ref. 12). This lends support to the suggestion (ref. 13) that the enzymatic reduction of intermediate **15** by tetrahydrofolate, is a radical reaction.

5-Methyltetrahydrofolate Model Mediated Carbon Transfer.

The enzyme methionine synthase (MS) has an essential requirement of the cofactor 5-methyl-tetrahydrofolate (**5**, ref. 14a). Two classes of MS are known; those which have a requirement of

cobalamine and those which are cobalamine-independent. In both cases the overall reaction involves the transfer of the N(5)-methyl substituent of the cofactor to the sulfur of the substrate amino acid homocysteine. An intriguing question concerns the activation of N(5)-methyl group for its transfer to either the sulfur atom of homocysteine or the cobalt of cobalamine. Plausible suggestions for this activation involve the creation of an electron deficient N(5)-centre, either by (one or two electron) oxidation or by its quarternization via coordination with a proximal electrophile (or H^+) located within the active site (ref. 14b). All attempts to transfer the methyl group of model **8**, under oxidative conditions, to the sulfur of a thiol substrate (PhSH) were unsuccessful. However, the N(5),N(5)-dimethylpterin system **17a** (Fig. 8) exhibited a facile transfer of one of the two methyl groups to the sulfur of phenyl thiol or homocysteine, resulting in the formation of phenyl methyl thioether or methionine, respectively (Fig. 8). This transfer was evidenced by employing the cofactor model **17b**, containing one $^{13}CH_3$ group. The products, namely, phenyl methyl thioether and methionine produced upon reaction of **17b** with phenyl thiol and homocysteine were both demonstrated (NMR) to possess a S-$^{13}CH_3$ group (ref. 15). Reaction of **17a** with cob(I)aloxime led to trace amounts of the transfer product to the cobalt system. The results of a systematic study of group transfer reactions from ammonium salts to thiols suggests that the mechanism of the reaction most likely involves radical intermediates (ref. 15).

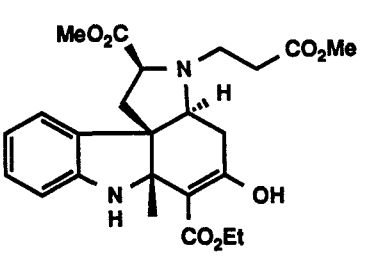


17a R = CH_3, **17b** R = $^{13}CH_3$

Fig. 8

Synthetic Methodology Based on Folate Models.

The natural cofactors have evolved to mediate the transfer of a one carbon unit in biological processes. Once it becomes possible to design cofactor models which can mimic the chemical function of the natural biomolecules, a whole new area of opportunities, based on group transfer from suitably tuned models, opens up for potential synthetic application. The lead reaction represented by the synthesis of tetrahydro β-carboline (Fig. 4), by transfer of the methylene group of model **10** to tryptamine, has been exploited by us in a general strategy for the synthesis of several heterocyclic systems related to indole alkaloids (ref. 4). In this context we have recently reported on the stereoselective synthesis of optically active system **18** (ref. 16) and on a short highly efficient synthesis of the pentacyclic C(21)β-isomer of the aspidosperma skeleton **19** (ref. 17, Fig. 9).



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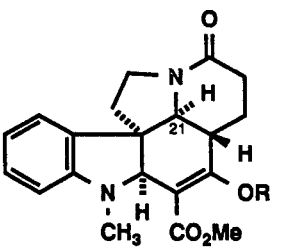

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Fig. 9

Acknowledgement. It is a pleasure to record my indebtedness to the coworkers, graduate students and technical assistants who have contributed to the chemistry of folate models and whose names appear in the appropriate cited references . This work was carried out in part under auspices of the Netherlands Foundation of Chemical Research (SON), with financial support of the Netherlands Organization of Scientific Research (NWO).

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