Stereochemistry of the Dihydrouracil Dehydrogenase Reaction in Metabolism of Uracil to β-Alanine

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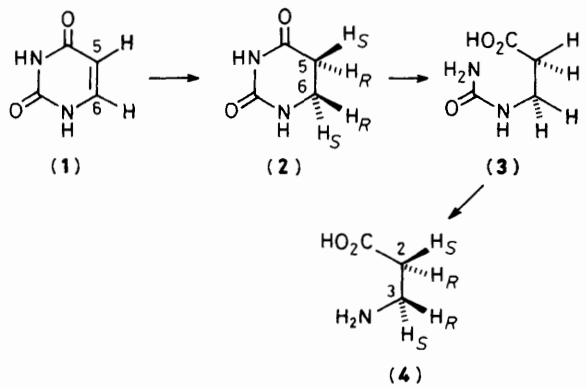
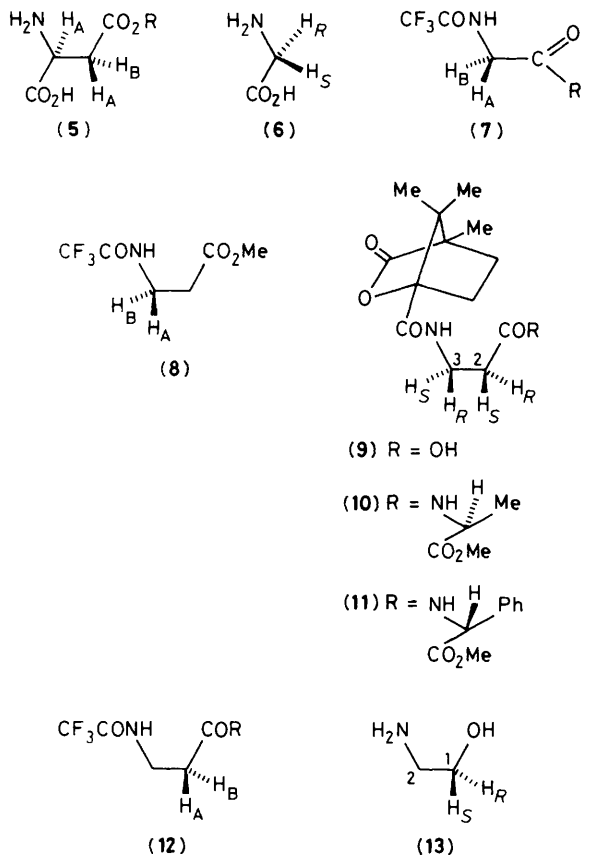
Samples of β-alanine stereospecifically labelled with deuterium in each of the four C–H bonds have been synthesised; these have been used to show that, in the first step of uracil metabolism, the pyrimidine is reduced by dihydrouracil dehydrogenase with overall *trans*-addition of hydrogen at the *si*-face at C-6 and the *si*-face at C-5.

Metabolism of the RNA base uracil (**1**) has been shown to occur in a variety of organisms by prior reduction to dihydrouracil (**2**) followed by hydrolysis, first to *N*-carbamoyl-β-alanine (**3**) and thence to β-alanine (**4**). The reaction (**1**) → (**2**), catalysed by dihydrouracil dehydrogenase (E.C. 1.3.1.2), is rate-limiting in this catabolic process and the enzyme has been shown to have decreased activity in cancer cells.[1] In order to assess the stereochemistry of this important rate-limiting step and to provide substrates for the study of other enzymic reactions involving β-alanine we decided to synthesise all four possible stereoisomers of [2-$^2H_1$]- and [3-$^2H_1$]-β-alanine and to provide an assay to differentiate between these compounds when isolated as products in the study of biochemical reactions.

(2*R*)[2-$^2H_1$]- and (2*S*,3*RS*)[2,3-$^2H_2$]-β-alanines were readily obtained from (2*S*,3*R*)[3-$^2H_1$]- and (2*S*,3*S*)[2,3-$^2H_2$]-aspartic acids [(**5**, R=H, H_B=^{2}H) and (**5**, R=H, H_A=^{2}H) respectively] which we have used in other studies.[2,3] These acids were converted into the corresponding β-methyl esters (**5**, R=Me)† by the method of Schwarz *et al.*[4] Decarboxylation by heating in the melt with *para*-methoxyacetophenone followed by hydrolysis with 6M-HCl at reflux yielded deuteriated β-alanines. An assay was now required to ensure that no racemisation had occurred during the synthesis.



† These compounds had the expected analytical and spectral properties.



For synthesis of the 3-deuteriated β-alanines, we used the commercially available enzyme L-alanine aminotransferase (E.C. 2.6.1.2),‡ known[5] to exchange the 2-*pro-R* hydrogen of glycine, to make (2*R*)[2-$^2H_1$]glycine (**6**, H_R=^2H) from glycine and ^2H_2O, and (2*S*)[2-$^2H_1$]glycine (**6**, H_S=^2H) from [2,2-$^2H_2$]-glycine. These samples were converted into the corresponding trifluoroacetamides (**7**, R=OH)† by reaction with trifluoroacetic anhydride. Reaction with $SOCl_2$ now afforded the crude acid chlorides (**7**, R=Cl) and these were converted into the diazoketones (**7**, R=CHN_2)† on treatment with diazomethane. Photolysis of the diazoketones in methanol afforded the methyl esters (**8**) which were hydrolysed directly to [3-$^2H_1$]-β-alanines. Since the Wolff rearrangement is expected[6] to occur with retention of configuration, even at a migrating primary chiral centre,[2] it was expected that the β-alanine obtained from (2*R*)[2-$^2H_1$]glycine (**6**, H_R=^2H) would be (3*R*)[3-$^2H_1$]-β-alanine (**4**, 3-H_R=^2H) and that from (2*S*)[2-$^2H_1$]glycine would be (3*S*)[3-$^2H_1$]-β-alanine (**4**, 3-H_S=^2H).§

Having ostensibly synthesised samples of β-alanine stereospecifically labelled with deuterium at all four C–H bonds, it was now necessary to assess the stereochemical integrity of these samples. This was readily achieved for the [3-$^2H_1$]-β-alanines by preparation of the amides (**9**) using camphanyl chloride. Parts of the 360 MHz ^1H n.m.r. spectra of these compounds are shown in Figure 1. It is evident from these that the synthesis has yielded optically pure (3*R*)- and (3*S*)-[3-$^2H_1$]-β-alanines. Unfortunately the region assigned to C-2 in the ^1H n.m.r. spectrum of (**9**) was not well enough resolved for us to differentiate the 2-*pro-R* and 2-*pro-S* hydrogens in this manner and attempts to improve the situation by introducing a second chiral centre as in the diamides (**10**) and (**11**) were unsuccessful even when lanthanide shift reagents were used.

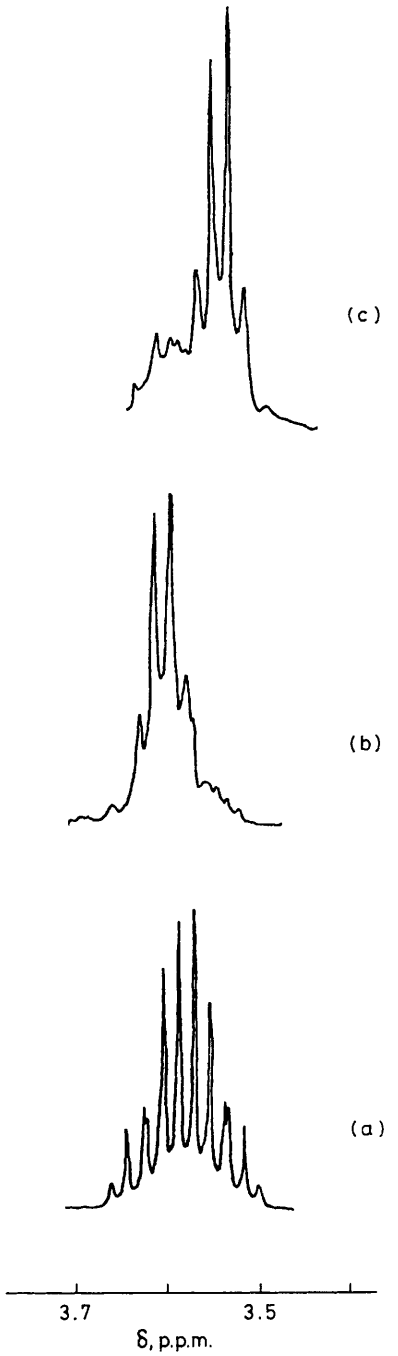


Figure 1. 360 MHz ^1H n.m.r. spectra ($CDCl_3$) of (a) the amide (**9**); (b) (3*R*)[3-$^2H_1$](**9**); and (c) (3*S*)[3-$^2H_1$](**9**).

In the event, the chirality of the [2-$^2H_1$]-β-alanines was assessed by converting them into (1*R*)- and (1*S*)-[1-$^2H_1$]-ethanolamines for which we had already developed an assay.[3] This was achieved by converting the β-alanines into the tri-

‡ Sold by Sigma Ltd. and Boehringer Mannheim as glutamate–pyruvate transaminase (E.C. 2.6.1.2.).

§ These assignments were checked by converting (3*S*)[3-$^2H_1$]-β-alanine into (2*S*)[2-$^2H_1$]ethanolamine by the method outlined here for the assay of the chirality at C-2 of β-alanine. The ^1H n.m.r. spectrum of the *N*,*O*-dicamphanic acid derivative correlated with that of the derivative of (2*S*)[2-$^2H_1$]ethanolamine prepared by $LiAlH_4$ reduction of methyl (2*S*)[2-$^2H_1$]glycinate.

fluoroacetamides (**12**, R=OH)† which were then converted *via* the acid chlorides (**12**, R=Cl) into the diazoketones (**12**, R=CHN_2).† Reduction with 55% *aqueous* HI in chloroform gave the ketones (**12**, R=Me)† which on Baeyer–Villiger oxidation using pertrifluoroacetic acid followed by hydrolysis gave the appropriate samples of ethanolamine (**13**). The ^1H n.m.r. spectra of the *N,O*-dicamphanic derivatives of these compounds showed that synthetic (2*R*)[2-$^2H_1$]-β-alanine had given (1*R*)[1-$^2H_1$]ethanolamine whilst synthetic (2*S*,3*RS*)-[2,3-$^2H_2$]-β-alanine had given (1*S*,2*RS*)[1,2-$^2H_2$]ethanolamine. This was the expected result and confirmed that the Baeyer–Villiger step in the assay had proceeded with retention of configuration at the migrating centre.

Having synthesised all four possible stereoisomers of [2-$^2H_1$]- and [3-$^2H_1$]-β-alanines and having devised assays for the identification of these compounds, we were now in a position to examine the stereochemistry of the reductive step in the metabolism of uracil. The enzymes of this process are quite ubiquitous in nature and have been reported to occur in beef liver.[7] Dihydrouracil dehydrogenase has recently been purified from rat liver and shown to contain FAD.[8] We adapted part of this latter purification procedure to obtain a partially purified mixture of enzymes from bovine liver. This mixture, together with NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase, was used to metabolise [5-$^2H_1$]- and [6-$^2H_1$]-uracils.[9] A similar incubation was conducted in ^2H_2O using uracil as substrate. Samples of β-alanine were isolated from all three experiments and these were subjected to the chirality assays devised above.

It was evident from the ^1H n.m.r. spectra of the *N*-camphanic acid derivatives that [6-$^2H_1$]uracil had been metabolised in H_2O to (3*R*)[3-$^2H_1$]-β-alanine (**4**, 3-H_R=^2H) whilst uracil in ^2H_2O had led to deuteriation at the 3-*pro*-*S* hydrogen. Metabolism of [5-$^2H_1$]uracil gave a sample of β-alanine which was converted into (1*R*)[1-$^2H_1$]ethanolamine. The metabolic product was therefore (2*R*)[2-$^2H_1$]-β-alanine (**4**, 2-H_R=^2H). There was insufficient sample from the ^2H_2O experiment to allow us to interpret the assay for the stereochemistry at C-2 with certainty.

It is evident from these results that, in the catabolism of uracil, the pyrimidine ring is reduced by dihydrouracil dehydrogenase with overall *trans*-addition of hydrogen. In this respect the reaction is a typical flavin-dependent redox reaction where *anti*-addition or removal of hydrogen is the rule.[10] The absolute stereochemistry of the process requires that hydrogen be added at the *si*-face at C-6 and the *si*-face at C-5.

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