Formation of *N*-Carbamoylaspartic Acid and Its Cyclisation to Orotic Acid

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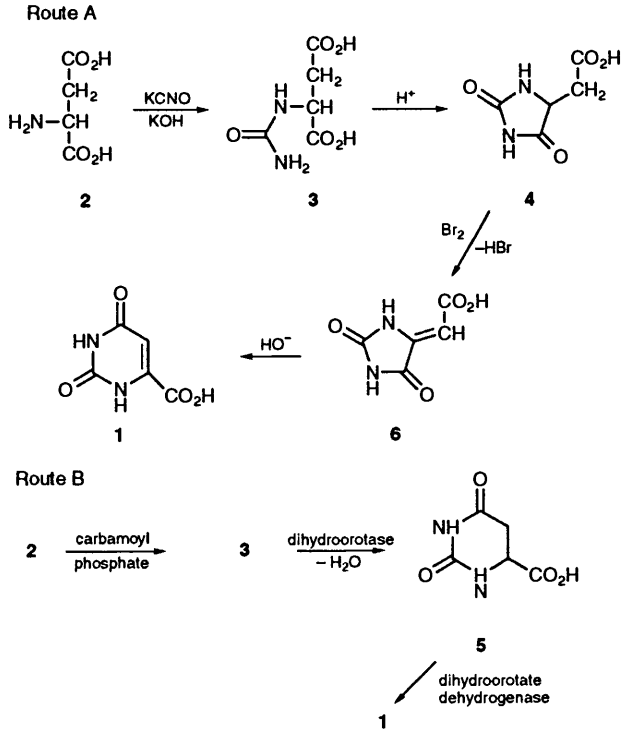
The stereochemistries of the reactions between cyanate and aspartic acid and 3-methylaspartic acid have been examined. The product of the first reaction, *N*-carbamoylaspartic acid **3**, can cyclise to give either dihydroorotic acid **5** or 5-carboxymethylhydantoin **4**. Acid catalysed cyclisation gives the latter while catalysis by the enzyme dihydroorotase gives the former. Introduction of a double bond into **3** changes the course of non-enzymatic cyclisation and a six-membered ring compound orotic acid **1** is the product. It is proposed that the double bond prevents intramolecular hydrogen bonding.

Orotic acid **1** is an important intermediate in the biosynthesis of pyrimidine nucleotides. It is possible to obtain **1** from aspartic acid **2** by two routes, one chemical (A)[1] and the other enzymatic (B)[2] (Scheme 1). In both pathways the first intermediate is *N*-



Scheme 1

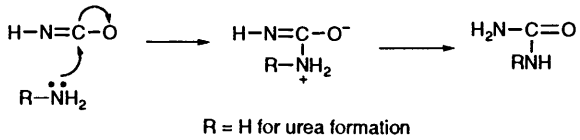
carbamoylaspartic acid **3**. In pathway A this cyclises to a five-membered ring compound 5-carboxymethylhydantoin **4** while enzymatically a six-membered ring compound, dihydroorotic acid **5**, is formed. Dehydrogenation of **4** gives a compound with an exocyclic double bond (5-carboxymethylidinehydantoin **6**) which, in alkaline solution, ring opens and spontaneously cyclises to a six-membered ring compound (orotic acid; **1**). In contrast, orotic acid is formed enzymatically by dehydrogenation of the six-membered ring compound **5**. We have made a number of experimental observations concerning some of the reaction steps which allow us to compare the chemical and biochemical pathways.

The conversion of **2** into **3** is identical to Wöhler's[3] synthesis of urea from ammonium cyanate (Scheme 2).

$$NH_4{}^+CNO^- \longrightarrow H_2NCONH_2$$

Scheme 2

The mechanism of urea formation, although studied by a number of workers, is not known with certainty. If the mechanism is ionic then the role of HO^- in the formation of **3** is difficult to understand. We repeated the preparation of **3** using HCl in place of KOH but only starting material was recovered. Shorter[4] has argued forcefully in favour of a molecular mechanism (Scheme 3), rather than an ionic one in the chemical

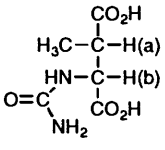


R = H for urea formation

Scheme 3

synthesis of urea, and this is consistent with our observations. The pK_a of cyanic acid[5] is 9.1 and that of the $-NH_3{}^+$ group of aspartic acid[5] is 10.0. Both are substantially greater than 7. Therefore, in the alkaline range there will be larger amount of the molecular species than there would be in the acid range and so reaction occurs in the presence of HO^- but not of HCl.

The stereochemistry of this reaction proved of some interest. Starting with (*R*)-aspartic acid ($[\alpha]_D = -32.12$) and (*S*)-aspartic acid ($[\alpha]_D = +28.16$), the product, which we thought would be (*R*)-*N*-carbamoylaspartic acid and (*S*)-*N*-carbamoylaspartic acid had $[\alpha]_D$ values of +0.85 and +0.37, suggesting extensive racemisation. This is surprising as the stereogenic centre in aspartic acid is not directly involved in the reaction. As the $[\alpha]_D$ values for the two forms of *N*-carbamoylaspartic acid are not known, it is difficult to assess the extent of racemisation which had occurred. However, we can ascertain this from a study of the analogous reaction of (2*S*,3*S*)-methylaspartic acid. This compound has two stereogenic centres and so racemisation gives a pair of diastereomers and this should be reflected in the NMR spectrum of the product **7**.



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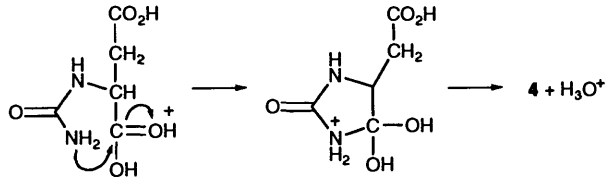
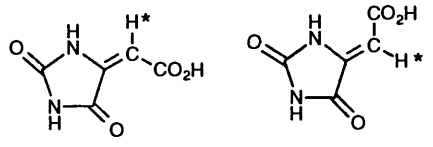
The spectrum of (2*S*,3*S*)-methylaspartic acid ($[\alpha]_D = +10.56$) in D_2O consists of a doublet (CH_3) at δ 1.25, a multiplet at δ 3.20 (H_a) and a doublet at δ 4.14 (H_b). The spectrum of the product **7** ($[\alpha]_D = -6.89$) in D_2O was much more complex: a double doublet at δ 1.40 (CH_3), a double multiplet at δ 3.35 (H_a) and a double doublet at δ 4.82 (H_b). The sets of peaks in each pair were of equal size, a result consistent with complete

racemisation at the 2-position. If the mechanism for the formation of **7** parallels that of urea in Scheme 3, racemisation should not occur. In general amino acids do not racemise in alkaline solution,[6] so that cannot be the explanation of the effect. However, *N*-acetylamino acids do racemise in alkaline solution because of the enhanced acidity of the methine group.[7] The carbamoyl group should have the same effect as the acetyl group and so racemisation occurs after formation of **3** and what we observed with both aspartic acid and with 3-methylaspartic acid is not inconsistent with the mechanism shown in Scheme 3.



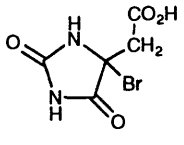
Scheme 4

Under acid conditions **3** cyclises to 5-carboxymethylhydantoin **4**. A probable mechanism is shown in Scheme 4. The mechanism of this step will be discussed in more detail later. Reaction of bromine with **4** and then dehydrobromination gives a compound with an exocyclic double bond **6**. The NMR spectrum suggests that it is a single compound, *i.e.* one of two geometrical isomers **6a** or **b**. In an attempt to decide between the



6a **6b**

two possibilities the product was subject to an NOE experiment. The NMR signal corresponding to the hydrogen marked with an asterisk was irradiated but this had no effect on either of the NH signals. Had the isomer been **6a** the H is close enough to the top NH for there to be an NOE effect. In the absence of an effect **6b** is more likely but this experiment does not fix the isomer with any degree of certainty. However, we can approach the problem another way. The bridgehead carbon is the one most likely to be brominated to give **8**.[8] Normal *anti* elimination should give **6b** and the absence of an NOE effect is consistent with the predicted pathway.



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The last step to be considered is the conversion of **6** into orotic acid in the presence of base. The kinetics of this process was studied in an excess of base and the rate law [eqn. (1)] was found to be:

$$\text{rate} = k_{obs}[\mathbf{6}] \quad (1)$$

A plot of k_{obs} *vs.* $[HO^-]$ is linear (Fig. 1) with no intercept and so the reaction is overall second order [eqn. (2)].

$$\text{rate} = k[\mathbf{6}][HO^-] \quad (2)$$

We suggest that base-catalysed ring-opening is the slow step

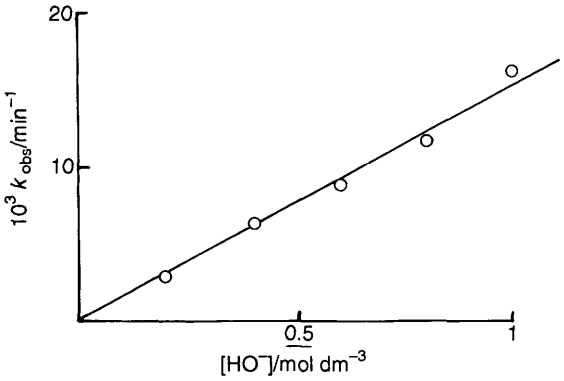
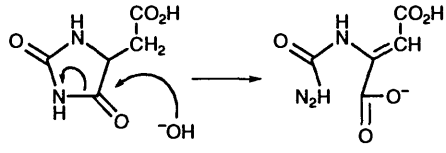


Fig. 1 Variatation of k_{obs} with $[HO^-]$ for the conversion of **6** into orotic acid



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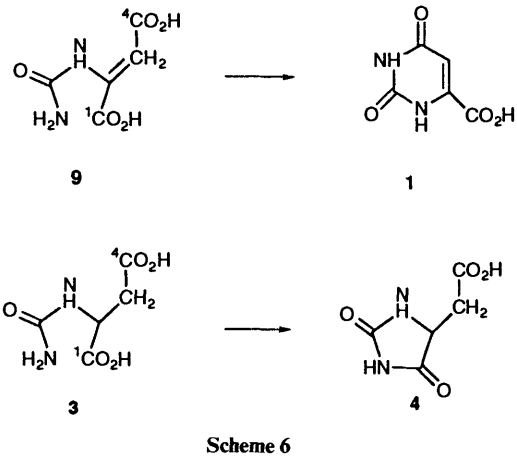
Scheme 5

(Scheme 5) followed by fast recyclisation at the other carboxylic acid group to give a six-membered ring compound.

We can now compare the mode of cyclisation of **3** and **9**, compounds which differ in that the latter possesses a double bond (Scheme 6). The formation of the hydantoin is straight-

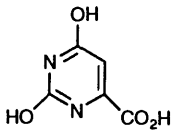


9 **1**

3 **4**

Scheme 6

forward as models indicate that the attacking NH_2 group is conveniently located to cyclise with the 1-carboxylic acid group. If the molecule were completely flexible it would be possible for the $-NH_2$ group also to approach the 4-carboxylic acid group with the potential for cyclisation. However, there is a strong possibility that the molecule is not completely flexible because of strong intramolecular hydrogen bonding between the two carboxylic acid groups. Molecular models show that this hydrogen bonding would not seriously impede approach of the NH_2 towards the 1-carboxylic acid group but it does lessen the possibility of cyclisation with the 4-carboxylic acid group. The presence of the double bond and the rigidity it introduces makes intramolecular hydrogen bonding between the carboxylic acid groups impossible and the NH_2 group can approach the 4-carboxylic acid group with the potential for cyclisation to give orotic acid **1**. When this can occur it is likely that it does so, as the product, in its enolic form (**10**), has some aromatic stabilisation. Clearly, the enzyme must bind the *N*-carbamoyl-



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aspartic acid in such a way that intramolecular hydrogen bonding is not possible, thus allowing cyclisation, *via* the 4-carboxylic acid group, to dihydroorotic acid rather than to **4**.

Experimental

Materials.—All reactants were reagent grade. The intermediates to orotic acid were prepared by the method of Nyc and Mitchell.[1] Potassium cyanate (8.1 g) and aspartic acid (13.4 g) were dissolved in KOH (1 mol dm^{-3}; 100 cm^3) and the mixture allowed to stand overnight. Addition of conc. HCl gave crystals of *N*-carbamoylaspartic acid (**3**) (9.4 g, 53%), m.p. 176 °C (lit.,[1] 179 °C); $\delta_C(D_2O)$ 39, 52, 177, 178 and 221. Compound **3** (9.4 g) was dissolved in 20% aq. HCl (40 cm^3) and evaporated to dryness. The residue was recrystallised (water) to give crystals of 5-carboxymethylhydantoin (**4**) (6.6 g; 77%), m.p. 212 °C (lit.,[1] 214 °C); *m/z* 158 (M^+); $\delta_C(D_2O)$ 37, 57, 162, 176 and 180. A mixture of bromine (1.28 g) and **4** (4.20 g) in glacial acetic acid (16 cm^3) was heated with shaking in a stoppered test tube at 100 °C for 4 h, during which the colour was discharged and a precipitate formed. After filtration the solid was recrystallised from water to give crystals of 5-(carboxymethylidine)hydantoin (**6**) (2.9 g; 71%); *m/z* 156 (M^+); ν_{max}/cm^{-1} 1680 (C=C) and 1730 (C=O); $\delta_H[(CD_3)_2SO]$ 5.6 (1 H, s) and 10.25 (1 H, s); $\delta_C[(CD_3)_2SO]$ 94.7, 140.3, 154.7, 164.4 and 166.6. Reaction of **6** (0.62 g) in KOH (1 mol dm^{-3}; 20 cm^3) for 3 h at 64 °C followed by acidification gave orotic acid (**1**), (0.51 g; 85%); m.p. > 320 °C (lit.,[1] 344 °C); $\delta_C[(CD_3)_2SO]$ 104, 144, 152, 163 and 165. (2*S*,3*S*)-Methylaspartic acid was a gift from Dr Catherine Botting, $[\alpha]_D = +10.56$ (c = 2.16, 5 mol dm^{-3} HCl). The *N*-carbamoyl derivative was prepared in the manner described above $[\alpha]_D = 6.89$ (c = 1.90, H_2O)

Instrumentation.—NMR spectra were obtained using a Bruker AM 300 spectrometer. Specific rotations were measured on an AA-1000 polarimeter.

Kinetic Studies.—Compound **6** was dissolved in KOH solution to give a solution of 10^{-3} mol dm^{-3} and absorbance changes at 255 nm monitored over 4 h. The first order rate constants were obtained graphically.

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