Mechanism of Acid Catalysis in the Cyclisation of 5-Aminolevulinic Acid and Acetylacetone to 3-Acetyl-4-(2-carboxyethyl)-2-methylpyrrole

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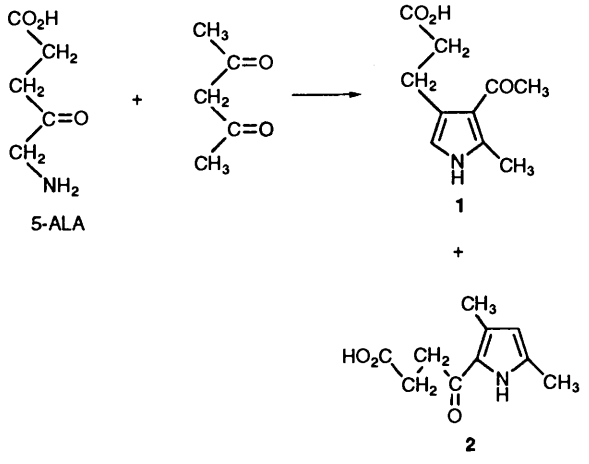
Under acid conditions 5-aminolevulinic acid reacts with acetylacetone to give 3-acetyl-4-(2-carboxyethyl)-2-methylpyrrole (**1**). There is also formation of a small amount of the Fischer–Fink product (**2**). Use of ^{13}C and ^{15}N NMR spectroscopy showed that the first condensation product to accumulate is the enaminoketone (**5**). The trifluoro analogue of **5** can be isolated and its cyclisation to **8** was monitored. There is a substantial spontaneous reaction and the acid-catalysed process occurs by specific acid catalysis.

5-Aminolevulinic acid (5-ALA) condenses with acetylacetone (pentane-2,4-dione) in an acid-catalysed reaction[1] to give a mixture of two products: 3-acetyl-4-(2-carboxyethyl)-2-methylpyrrole **1**, the Knorr product, and 2-(3-carboxypropionyl)-3,5-dimethylpyrrole **2**, the Fischer–Fink product (Scheme 1). The



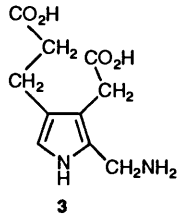
Scheme 1

former is the dominant product, forming 95% of the total. We are interested in this reaction as it is a nonenzymic model for the enzyme-catalysed condensation[2] of two molecules of 5-ALA to form prophobilinogen **3**, a buildng block *en route* to a number of important natural pigments. The reaction shown in Scheme 1 involves several bond-breaking and bond-making processes. As the reaction occurs only under acid conditions, acid catalysis must play an important role in effecting reaction but not all the steps need be acid-catalysed; some could occur spontaneously once a certain reaction intermediate has been formed. In a previous publication[3] we proposed a sequence of steps in the formation of **1** and **2** and now report an attempt to identify the catalysed steps within that sequence.

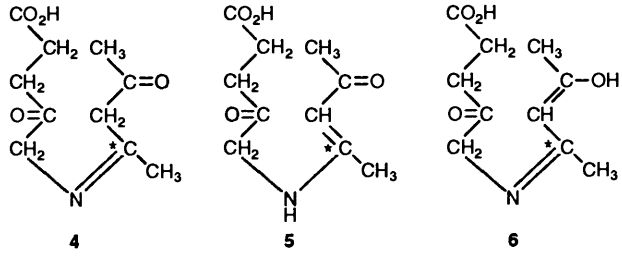
The Fischer–Fink product **2** is formed in such small quantities that we have made no attempt to delineate the mechanism of its formation. Factors influencing the relative amounts of Knorr and Fischer–Fink products have been discussed previously.[3]

Results and Discussion

It is necessary, firstly, to establish the tautomeric form of the intermediate formed by the initial condensation of 5-ALA with



pentane-2,4-dione. It could be a ketimine **4**, an enaminoketone **5**, or an enimine **6**. The reaction of a 1:1 mixture of [4-^{13}C]-



ALA·HCl (5% enriched) and pentane-2,4-dione in an acetate buffer containing deuterioacetic acid of pH 6.45 was monitored over 6 h and a spectrum recorded every 20 min. An examination of the spectra revealed that, apart from the reactant peaks which diminished in intensity with time and the product peaks which increased in intensity with time, there was a cluster of peaks which grew to a maximum and gradually diminished to zero as the reaction proceeded. The transient peaks within the cluster were assigned to a single intermediate species since they all appear to grow to a maximum and diminish at the same time. Most of the peaks in the cluster are similar to those in the reactants, as would be expected, but there is one peak at δ 167.1 which lies in the ^{13}C NMR shift range for imines and enamines.[4] This observation, however, does not allow us to distinguish between the three possibilities **4**–**6**. The carbon atom marked with an asterisk, which is the location of the label, would have a similar shift in all three. The appearance of a signal with this shift, however, does indicate that it is the unsaturated intermediate, rather than the previously formed aminoalcohol, which accumulates, *i.e.* dehydration of the aminoalcohol is fast.

A more detailed examination of the spectral changes occurring during reaction yielded little extra information. They were recorded over only 6 h, although the reaction was incomplete at this stage, as the products saturate the reaction mixture and come out of solution. As a result, uniform shimming could not be attained and no significant changes

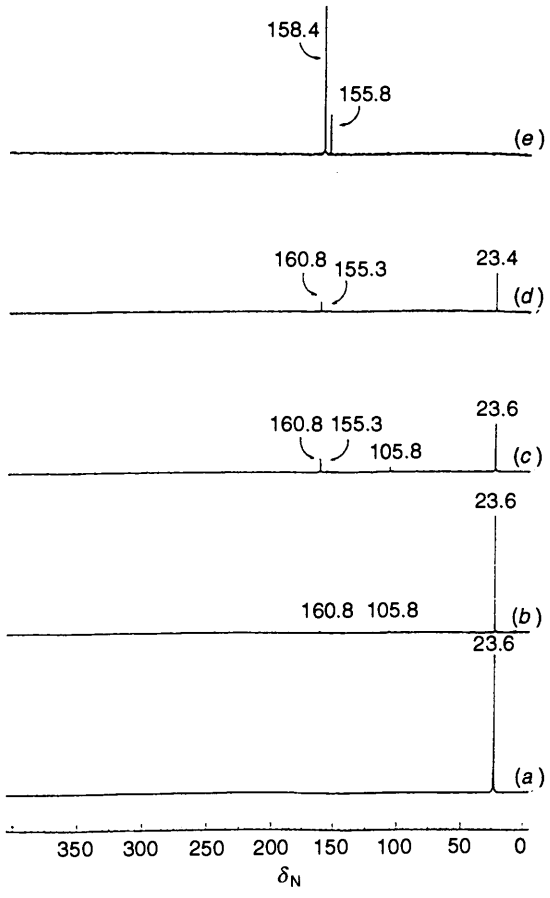


Fig. 1 ^{1}H Decoupled 30.142 MHz ^{15}N NMR spectra of a mixture of [^{15}N]-5-ALA and pentane-2,4-dione in acetate buffer: (*a*) on mixing; (*b*) after 7 min; (*c*) after 25 min; and (*d*) after 105 min

were observed after 6 h. The ^{13}C NMR spectroscopic study was repeated with four other buffer of pHs 5.87, 5.40, 5.07 and 4.59 but no intermediates, other than that observed in the first experiment, were observed. However, it was noted that the reaction was slowed by a lowering of the pH. This is most readily described to increased protonation of the amino-group of 5-ALA with consequent decrease in its nucleophilicity. The experiment was also repeated using 50% enriched [4-^{13}C]-5-ALA in the hopes of detecting any intermediates accumulating at very low concentrations but nothing new was observed. The fact that a linear intermediate of the type **4–6** does accumulate at a level which permits detection by NMR spectroscopy suggests that the subsequent cyclisation step is the slowest in the overall reaction and that this situation is rather insensitive to pH.

To distinguish between **4**, **5** and **6** as the accumulating intermediate we used ^{15}N NMR spectroscopy. The ^{15}N chemical shift ranges, with respect to external, neat nitromethane (δ 380.23), of the relevant chemical species are as follows:[5] amine hydrochlorides (15–75 ppm), enaminoketones (80–125 ppm), pyrroles (125–165 ppm) and imines (305–365 ppm). The separation of the values for enaminoketones and imines allows us to distinguish between them with ease. A reaction mixture which was equimolar (1.5 mmol dm^{-3}) in [^{15}N]-5-ALA (50% enriched) and pentane-2,4-dione was monitored by ^{15}N NMR spectroscopy over 2 h and spectra recorded at timed intervals (Fig. 1). The signal due to the amino-group of 5-ALA at 23.6 ppm decreased monotonically with time and that due to the pyrrole nitrogen of **1** at 160.8 ppm increased in like manner. A small peak at 105.8 ppm reached a maximum 25 min after mixing and then disappeared. This we

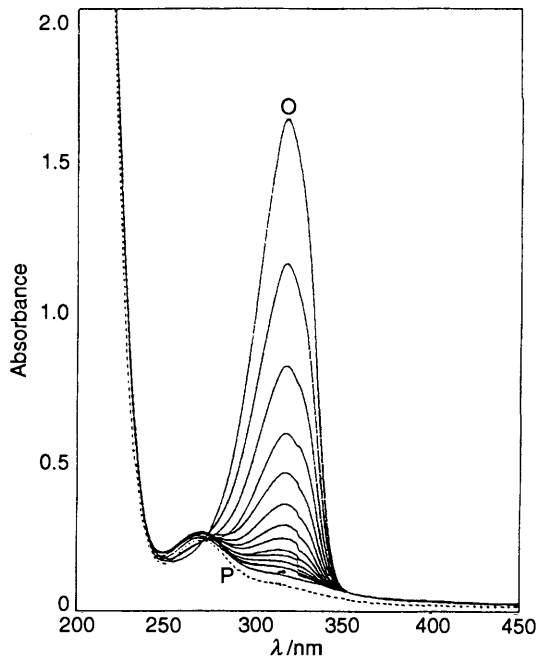
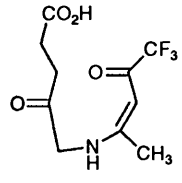


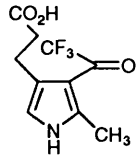
Fig. 2 Spectral changes accompanying the conversion of **7** into **8** in acetate buffer at 40 °C

ascribe to the enaminoketone **5** and so the tautomeric form of the transient intermediate has been identified. However, this does not completely rule out a ketimine or enimine as the species formed by dehydration or as the reactive species for subsequent cyclisation. It may form and then tautomerise to the more stable enaminoketone. Intramolecular hydrogen bonding may well favour the enaminoketone.

As reported previously,[3] in the reaction of 5-ALA with 1,1,1-trifluoropentane-2,4-dione, the enaminoketone **7** was actually



7



8

isolated and identified. Refluxing **7** in acetate buffer results in the formation of the Knorr pyrrole **8**. The fact that formation of both **8** and **1** appears to involve an enaminoketone intermediate suggests that any conclusions deduced for the mechanism of the formation of **8** would also apply to the formation of **1**.

As **7** could be readily isolated from the reaction mixture we were able to study its cyclisation to **8**, a much simpler matter than having to start with 1,1,1-trifluoropentane-2,4-dione. A solution of **7** in acetate buffer has an intense UV absorption of 317.6 nm and at 40 °C there is a gradual change in the spectrum (Fig. 2) until it becomes identical with that of **8** in acetate buffer. These data allowed us to monitor the cyclisation reaction as a function of pH and the results are displayed in Fig. 3, where k_{obs} is the pseudo-first-order rate constant for the cyclisation reaction. The linearity of the plot and the presence of an intercept means that the data can be accommodated in the rate law [eqn. (1)], when k_o, the rate constant for the

$$k_{obs} = k_o + k_H[H_3O^+] \quad (1)$$

spontaneous, uncatalysed process, is 0.10 h^{-1} and k_H, the rate

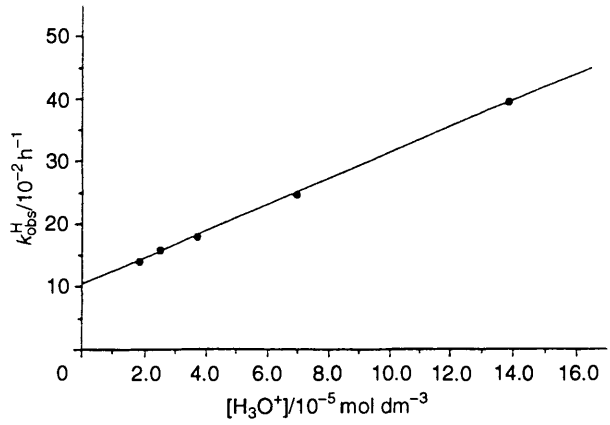


Fig. 3 Variation of k_{obs} with pH for the conversion of **7** into **8**

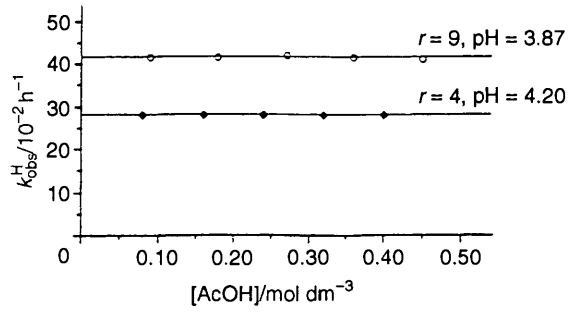


Fig. 4 Effect of buffer concentration at constant pH for the conversion of **7** into **8**

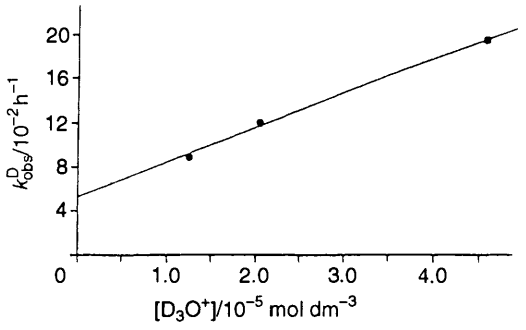


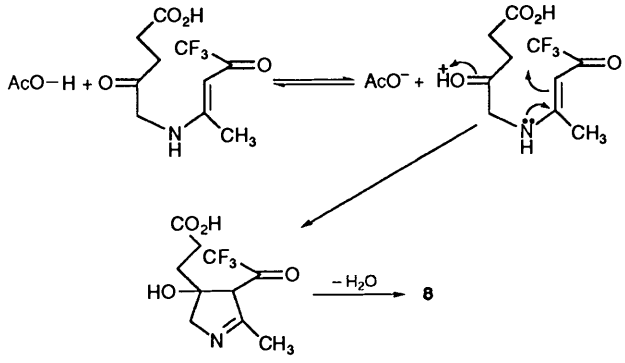
Fig. 5 Variation of k_{obs} with pD for the conversion of **7** into **8**

constant for the hydronium ion catalysed process, is 2.15×10^3 dm^3 mol^{-1} h^{-1}. To investigate the possibility of general acid catalysis the variation of k_{obs}, at two pHs, as a function of buffer concentration was determined. The results are displayed in Fig. 4 and indicate clearly that general acid catalysis does not occur.

As the spontaneous or water reaction is so large, we studied the kinetics of cyclisation in pure water. No acid is produced during cyclisation and so buffering is not important for that reason. Addition of a small quantity of **7** to water in a cuvette produced the same spectral changes as shown in Fig. 2. The values measured for the decrease in absorbance at 317.6 nm fit a first order rate law to give a value of k_o of 0.10 h^{-1}, identical with that obtained from the plot in Fig. 3. Replacing isotopically normal water with heavy water gave a k_o value of 0.052 h^{-1} and thus the kinetic isotope effect for the spontaneous reaction is 1.98.

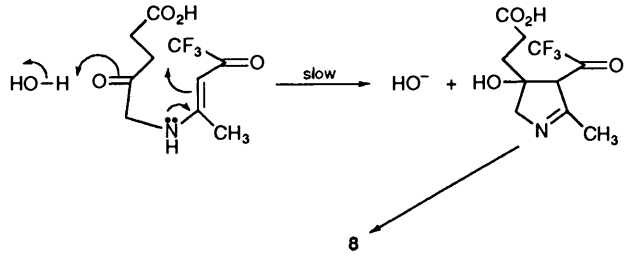
A number of deuteriated buffers were prepared and the rate of cyclisation in them measured. A plot of k_{obs} *vs.* $[D_3O^+]$ is shown in Fig. 5. The intercept (0.052 h^{-1}) is identical with the value of k obtained for cyclisation in pure D_2O. The slope (k_D) is 3.1×10^3 dm^3 mol^{-1} h^{-1} and so the kinetic isotope effect for the acid-catalysed cyclisation is 0.69. This inverse isotope is consistent with a mechanism involving equilibrium protonation preceding slow cyclisation (**4**–**6**). For the deuteriated buffers the concentration of protonated intermediate will be greater than that in an isotopically normal buffer, leading to a rate enhancement. Protonation of the carbonyl group could be a model for the type of electronic polarisation which is induced by the enzyme in the cyclisation of 5-ALA to form porphobilinogen.

The kinetic isotope effect for the spontaneous reaction is consistent with the mechanism shown in Scheme 2. The driving



Scheme 2

force in the cyclisation reaction is, of course, the aromatic stabilisation of the pyrrolic product. For reasons given above, we feel that the mechanisms of formation of **1** from 5-aminolevulinic acid and acetylacetone is likely to similar to those shown in Schemes 2 and 3.



Scheme 3

Experimental

Materials.—The reagents were laboratory grade or AnalaR. The syntheses of ^{13}C- and ^{15}N-labelled 5-ALA has already been described.[6]

Instrumentation.—The kinetics using ^{13}C NMR spectroscopy were performed at 300.134 MHz on a Bruker AM 300 spectrometer using the kinetics program AV KINETICS.AVR. Spectra were acquired using 32 K data points, a pulse width of 2.0 μs (3.9 μs for a 90° flip angle), a recycle time of 1.82 s and a spectral width of 20 000 Hz. The probe temperature was maintained at 21 °C. Each accumulation, with a scan time of 921 s, was preceded by 4 dummy scans (9 s) and followed by a delay time of 260 s. Thus, the time between the start of successive accumulations was 12 00 s (20 min). Spectra were referenced with respect to internal CD_3OD set at δ 49.04. They were processed after the peak intensities of the first spectrum were normalised, by setting the absolute intensity parameter to unity before the spectrum was transformed. This ensured that peak intensities of each successive spectrum were in relation to those of the first. The data for the spectra were analysed on an Apple Mac using a BASIC ^{13}C data handling program devised by Broan.[7]

The kinetics of cyclisation of **7** was followed spectrophotometrically with a Philips PU8732 UV–VIS scanning spectrophotometer, fitted with a PU8732 cell programmer. The latter was equipped with a thermostated multicell compartment in which the temperature was maintained at 40 ± 0.1 °C by circulating water. The reaction was followed by monitoring the disappearance of the peak at 317.6 nm and infinity values were recorded after ten half-lives. First-order plots were obtained manually on graph paper.

All pH measurements were made at 40 °C with a Griffin digital pH meter equipped with a PHP-100-030C glass electrode. A correction of 0.40 pH units was added to the meter reading to obtain pD values.[8] The pH meter was checked against a standard of known pH before each measurement.

Acknowledgements

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