A Kinetic Analysis of the Acidic Degradation of Penicillin G and Confirmation of Penicillamine as a Degradation Product

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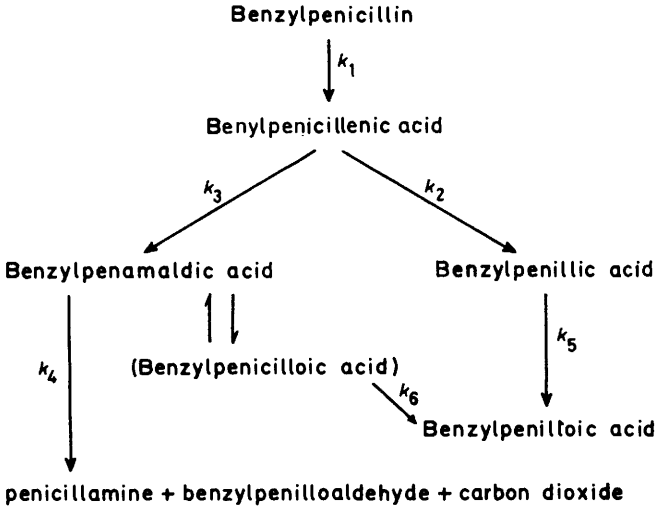
Two recently proposed degradation pathways for the acidic degradation of pencillin G were examined by calculating the theoretical time course based on each pathway and examining the fit of the theoretical predictions to the experimental data. A substantial difference in the goodness of fit was observed. The degradation pathway which provided the best fit included penicillamine as a terminal degradation product. This pathway is therefore favoured since penicillamine was also identified as a degradation product of penicillin G by both reversed-phase high pressure liquid chromatography and differential pulse polarography.

In 1976 a pathway for the acidic degradation of penicillin G was proposed based upon the anion-exchange high pressure liquid chromatographic (h.p.l.c.) analysis of penicillin G solutions aged at pH 2.7.[1] Although this degradation pathway is similar to previously proposed schemes,[2,3] it is unique in that it is based totally on the identification and quantification of the degradation products which appear in penicillin G solutions during ageing. Several potential degradation pathways were fitted to the experimental data and the best fit for the data was proposed as the degradation pathway in acidic media (Scheme 1).

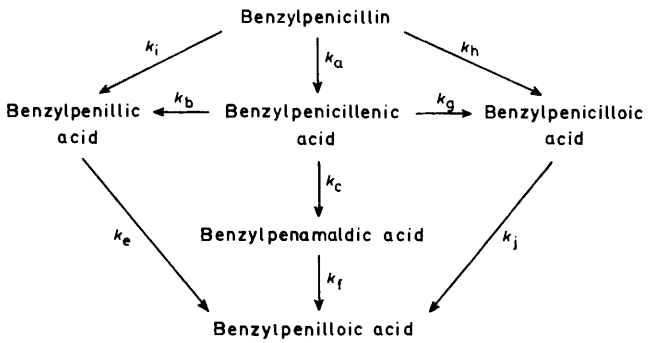


Scheme 1

Recently another degradation pathway was proposed which is based upon n.m.r. measurements of penicillin G solutions during ageing at pH 2.5.[4] This degradation pathway (Scheme 2) differs from Scheme 1 in two major aspects: (1) penicillin G is degraded by three parallel reactions rather than directly to benzylpenicillenic acid; (2) penicillamine and benzylpenilloaldehyde are not formed.

The purpose of this report is to clarify the uncertainty which presently exists concerning the acidic degradation pathway of penicillin G.

The first cited difference between Schemes 1 and 2 was investigated by calculating the theoretical time course for the acidic degradation of penicillin G based on Scheme 2 and examining the fit of the theoretical behaviour to the n.m.r. analytical data. Schemes 1 and 2 were investigated by examining aged, acidic penicillin



Scheme 2

G solutions for the presence of penicillamine by a reversed-phase h.p.l.c. system and by a differential pulse polarographic method which has been shown to be specific for penicillamine.[5,6]

EXPERIMENTAL

Potassium penicillin G and D-penicillamine were obtained commercially. Potassium penicillin G (0.05M) was dissolved in a 0.1M-phosphate buffer, pH 2.5, and aged at 37 °C. Samples were analysed by reversed-phase h.p.l.c. (ALC 202 and μ-Bondapak C18; Waters Associates) utilizing a u.v. detector at 254 nm. The mobile phase was composed of 10% acetonitrile and 90% 0.06M-phosphate buffer at pH 4.5. The flow rate was 0.8 ml min^{-1} and the u.v. attenuation was 0.16 AUFS (absorbance units full scale). A differential pulse polarographic (d.p.p.) method was used which had been found to be specific for penicillamine.[6] The supporting electrolyte was 0.056M-citrate buffer at pH 6.45 containing 0.006% Triton X-100 (scintillation grade; Eastman Kodak). Samples of the aged penicillin G solution were spiked with reference penicillamine and immediately analysed by reversed-phase h.p.l.c. and d.p.p.

RESULTS AND DISCUSSION

The system of differential equations which describes Scheme 2 was integrated analytically and the solution

was programmed for a digital computer. Although the rate constants associated with Scheme 2 are not explicitly stated in ref. 4, it is possible to deduce the value of k_a, k_b, k_g, k_h, and k_i. The rate constant for the loss of penicillin G is the sum of k_a, k_i, and k_h and is reported as 0.44 min^{-1}.[4,*] The proportions of penicillin G which are degraded through benzylpenicillenic acid, benzylpenicilloic acid, and benzylpenillic acid are reported as 50 : 13 : 37.[4] The rate constants for the conversion of penicillin G to each of these degradation products must also be in this ratio, *i.e.*, k_a 0.22, k_i 0.16, and k_h 0.06 min^{-1}.

The rate of disappearance of benzylpenicillenic acid in Scheme 2 is reported to be identical to the rate of disappearance of benzylpenicillenic acid in Scheme 1.[4] The rate constant for the loss of benzylpenicillenic acid in Scheme 1 is 0.7 min^{-1}, *i.e.*, the sum of k_2 and k_3. Thus in Scheme 2, the sum of k_b, k_c, and k_g is also 0.7 min^{-1}. The proportions of benzylpenicillenic acid which are degraded through benzylpenillic acid, benzylpenamaldic acid, and benzylpenicilloic acid are 20 : 24 : 6 and the rate constants must be in this same ratio, *i.e.*, k_b 0.28, k_c 0.34, and k_g 0.08 min^{-1}.

The rate of appearance of benzylpenicillenic acid in Scheme 2 is stated [4] to be equal to the rate of appearance of benzylpenicillenic acid in Scheme 1 which is 0.031 min^{-1}. However, determination of k_a based on the rate of loss of penicillin G led to the earlier conclusion that $k_a = 0.22$ min^{-1}. Scheme 2 is analysed using both values of k_a. Thus Scheme 2A uses k_a 0.22 min^{-1} and Scheme 2B k_a 0.031 min^{-1} to test the fit of the n.m.r. data.

No direct information is given [4] regarding the value of k_e, k_f, or k_j in Scheme 2. However, values for these rate constants were assigned by assuming that the formation of benzylpenilloic acid follows apparent first-order kinetics and that k_e, k_f, and k_j are in the same proportion as the total concentrations of benzylpenillic acid, benzylpenamaldic acid, and benzylpenicilloic acid, *i.e.*, 57 : 24 : 19. The combined rate constant for the formation of benzylpenilloic acid (the sum of k_e, k_f, and k_j) was set equal to the slope of a plot of ln([benzylpenilloic acid]) *versus* time. When the n.m.r. data for benzylpenilloic acid is plotted in this manner, the regression equation has an R^2 value of 0.91 indicating an acceptable linear fit and the slope is 1×10^{-4} min^{-1}. Thus the assumptions lead to the conclusion that $k_e = 5.7 \times 10^{-5}$, $k_f = 2.4 \times 10^{-5}$, and $k_j = 1.9 \times 10^{-5}$ min^{-1}.

An alternative approach for the assignment of values of k_e, k_f, and k_j is to assume that they have the same value as equivalent reactions in Scheme 1. Thus, 1.2×10^{-4} min^{-1}, 5.0×10^{-4} min^{-1}, and 0.0 were used for k_e, k_f, and k_j, respectively. The fit of the n.m.r. data to Scheme 2 did not change significantly when this assumption was used.

The rate constants associated with Schemes 1, 2A, and 2B are listed in the Table. The values of the rate constants for corresponding reactions in Schemes 1 and 2 are very similar considering that the experimental conditions were not identical, *i.e.*, pH 2.7 *versus* 2.5 and 0.0367M-buffer *versus* no buffer.

Rate constants (min^{-1}) associated with Schemes 1 and 2

Rate constant from Scheme 1	Corresponding rate constant from Scheme 2A	Corresponding rate constant from Scheme 2B
k_1 3.1×10^{-2}	k_a 2.2×10^{-1}	k_a 3.1×10^{-2}
k_2 5.0×10^{-1}	k_b 2.8 $\times 10^{-1}$	k_b 2.8 $\times 10^{-1}$
k_3 2.0×10^{-1}	k_c 3.4 $\times 10^{-1}$	k_c 3.4 $\times 10^{-1}$
k_4 2.3×10^{-4}		
k_5 1.2×10^{-4}	k_e 5.7×10^{-5}	k_e 5.7×10^{-5}
k_6 5.0×10^{-4}	k_f 2.4×10^{-5}	k_f 2.4×10^{-5}
	k_g 8.0×10^{-2}	k_g 8.0×10^{-2}
	k_h 6.0×10^{-2}	k_h 6.0×10^{-2}
	k_i 1.6×10^{-1}	k_i 1.6×10^{-1}
	k_j 1.9×10^{-5}	k_j 1.9×10^{-5}

The mean square absolute deviations (absolute deviation is the absolute value of observed value minus predicted value) for the n.m.r. data with Schemes 2A and 2B are 2.87 and 2.62, respectively, indicating that values of k_a as different as 2.2×10^{-1} and 3.1×10^{-2} min^{-1} have little effect on the fit of the n.m.r. data to Scheme 2.

Figures 1—7 compare the two different sets of experi-

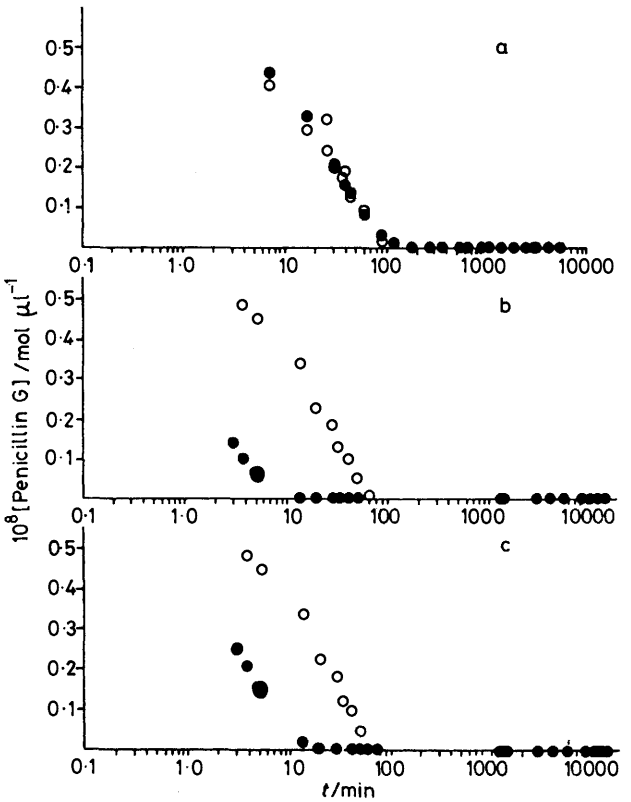


FIGURE 1 Comparison of analytical concentration of penicillin G, ○, to predicted concentration, ●. a, H.p.l.c. data with Scheme 1; b, n.m.r. data with Scheme 2A; c, n.m.r. data with Scheme 2B

* A personal communication [7] received after this manuscript was submitted noted that 0.44 min^{-1} is not the intended value. In further work we have optimized the fit of the proposed Schemes to the respective experimental data. Some inconsistencies still exist between the optimized constants and Scheme 2 (even with the intended value), which will be discussed in a manuscript now in preparation.

mentally determined concentrations of penicillin G and its degradation products to theoretically predicted concentrations. The h.p.l.c. data at pH 2.7, 37 °C, and 0.367M-buffer [1] is compared to the theoretical values

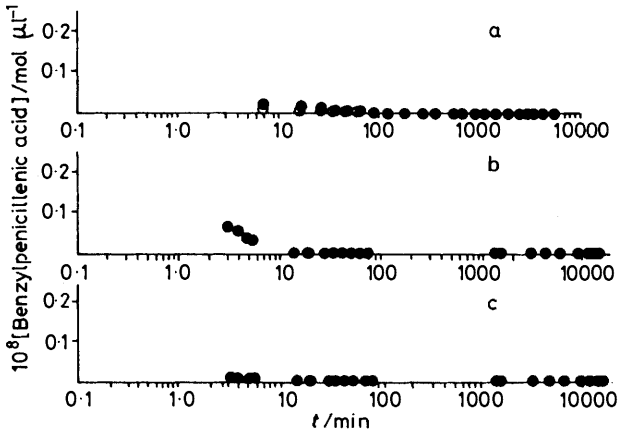


FIGURE 2 Comparison of analytical concentration of benzylpenicillenic acid, ○, to predicted concentration, ●. Note that a u.v. assay procedure was used to quantify benzylpenicillenic acid in the h.p.l.c. data while the n.m.r. data did not quantify benzylpenicillenic acid. a, H.p.l.c. data with Scheme 1; b, predicted values by Scheme 2A; c, predicted values by Scheme 2B

obtained from Scheme 1 while the n.m.r. data at pH 2.5, 37 °C, and no buffer [4] is compared to the theoretical values obtained from Schemes 2A and 2B.

Inspection of Figures 1a—4a show that the h.p.l.c.

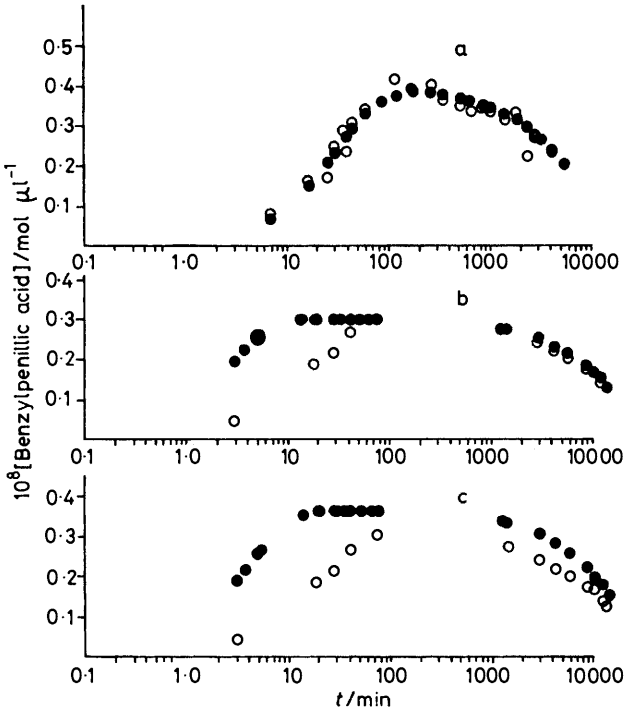


FIGURE 3 Comparison of analytical concentration of benzylpenillic acid, ○, to predicted concentration, ●. a, H.p.l.c. data with Scheme 1; b, n.m.r. data with Scheme 2A; c, n.m.r. data with Scheme 2B

data for penicillin G, benzylpenicillenic acid, benzylpenillic acid, and benzylpenamaldic acid coincides with the theoretical values predicted by Scheme 1. The actual concentration of penicillamine (Figure 5a) and benzylpenilloic acid (Figure 6a) were slightly greater than predicted by Scheme 1.

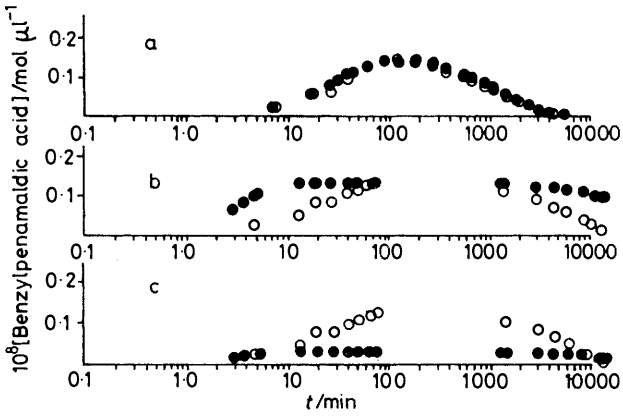


FIGURE 4 Comparison of analytical concentration of benzylpenamaldic acid, ○, to predicted concentration. ●, a, H.p.l.c. data with Scheme 1; b, n.m.r. data with Scheme 2A; n.m.r. data with Scheme 2B

The concentrations predicted by Schemes 2A and 2B do not coincide with the n.m.r. data for penicillin G or any of the degradation products. Scheme 2A underestimates the n.m.r. values for penicillin G (Figure 1b) and benzylpenilloic acid (Figure 6b) while overestimating the values for benzylpenillic acid (Figure 3b), benzylpenamaldic acid (Figure 4b), and benzylpenicilloic acid (Figure 7b). Scheme 2B underestimates the n.m.r. values for penicillin G (Figure 1c), benzylpenamaldic acid (Figure 4c), and benzylpenilloic acid (Figure 6c)

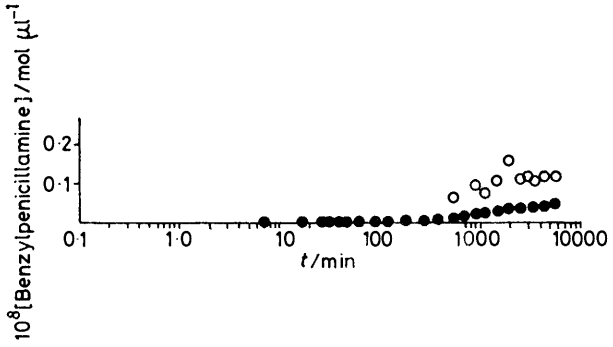


FIGURE 5 Comparison of analytical concentration of penicillamine determined by h.p.l.c., ○, to concentration predicted by Scheme 1. Note that the n.m.r. analysis did not detect any penicillamine and it is not included in Scheme 2

while overestimating the values for benzylpenillic acid (Figure 3c) and benzylpenicilloic acid (Figure 7c). The assumptions made in order to assign values to k_e, k_t, and k_j are not responsible for the poor fit as penicillin G, whose concentration is independent of k_e, k_t, or k_j, was underestimated by both Scheme 2A and 2B.

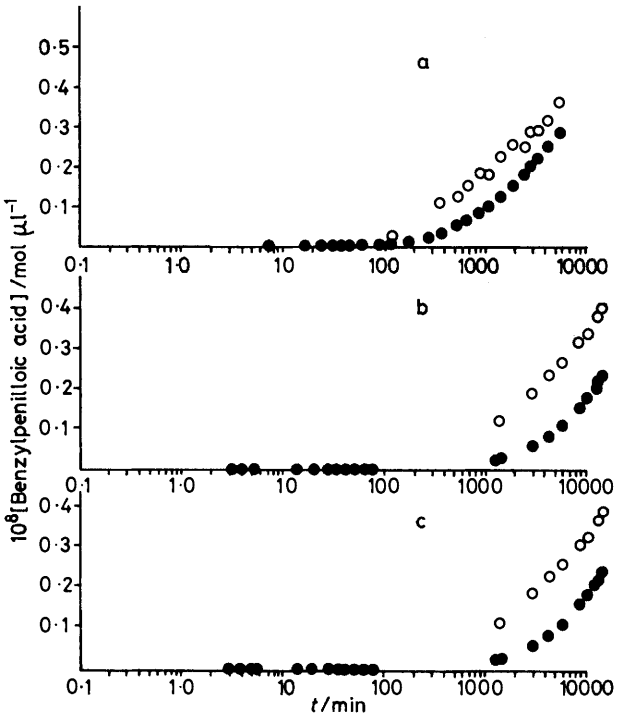


FIGURE 6 Comparison of analytical concentration of benzylpenilloic acid, ○, to predicted concentration, ●. a, H.p.l.c. data with Scheme 1; b, n.m r data with Scheme 2A; c, n.m.r. data with Scheme 2B

It is interesting to observe Figure 8 which shows that the n.m.r. data fits the values predicted by Scheme 1 significantly better than either Scheme 2A or 2B. Thus it is clear that the degradation profile of penicillin G generated by Scheme 1 describes the actual degradation of penicillin G more accurately than either Scheme 2A or 2B.

Penicillamine formation during the acidic degradation

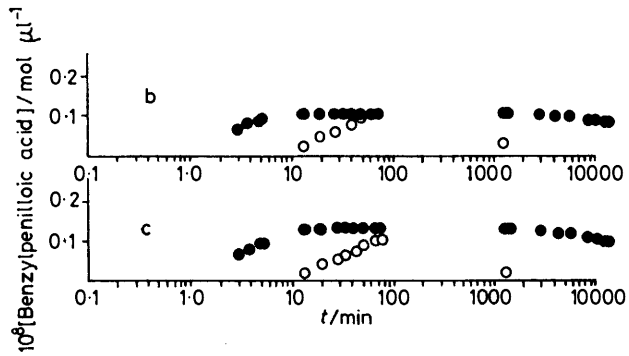


FIGURE 7 Comparison of analytical concentration of benzylpenilloic acid determined by n.m.r., ○, to concentration predicted by Scheme 2A, top, and Scheme 2B, bottom. Note that the h.p.l.c. analysis did not detect any benzylpenicilloic acid

of penicillin G sharply distinguishes Scheme 1, in which penicillamine is a terminal degradation product, from Scheme 2 which does not include penicillamine. That penicillamine actually occurs as a product was verified by use of a reversed-phase h.p.l.c. system rather than the anion-exchange h.p.l.c. system originally employed to identify penicillamine.[8]

A chromatogram of 0.05M-penicillin G which was aged

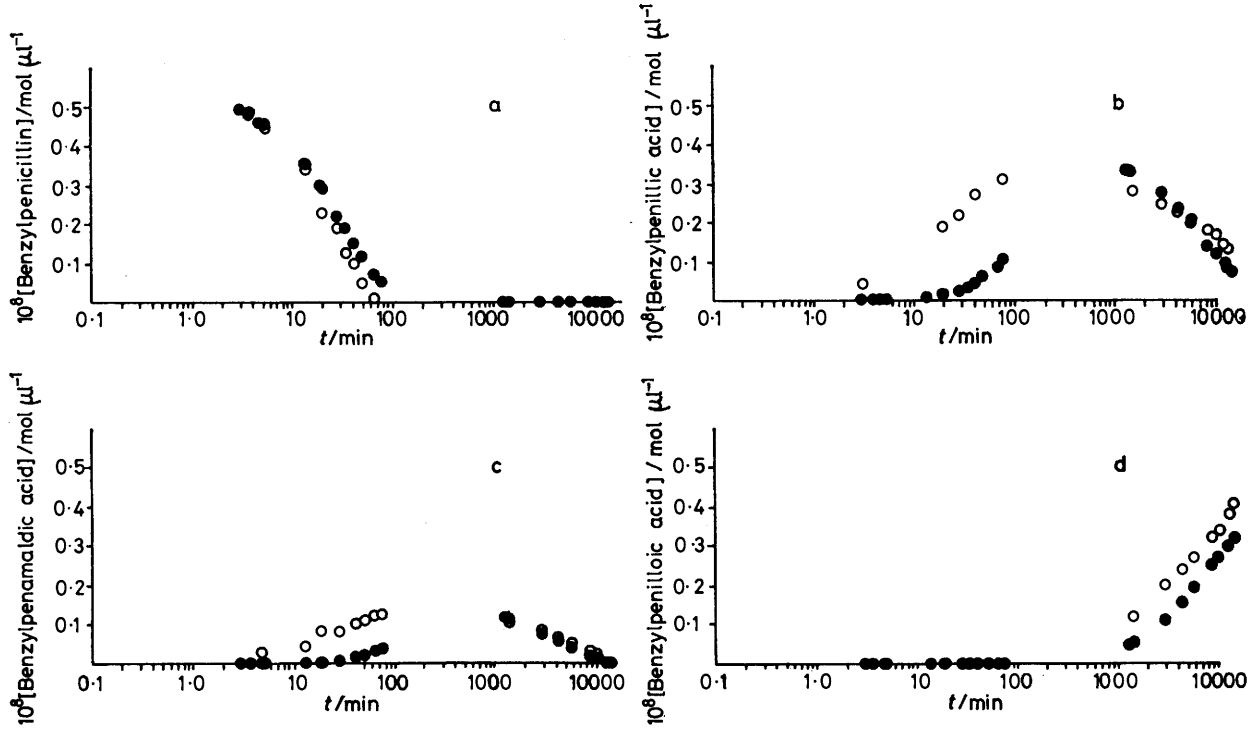


FIGURE 8 Comparison of analytical concentration determined by n.m.r., ○, to predicted concentration based in Scheme 1, ●. a, Penicillin G; b, benzylpenillic acid; c, benzylpenamaldic acid; d, benzylpenilloic acid

for three days at 37 °C in 0.1M-phosphate buffer (pH 2.5) is shown in Figure 9a. The peak at 4.7 min corresponds to the retention time of reference penicillamine. To confirm that this peak is penicillamine, a portion of the aged penicillin G solution was spiked with reference penicillamine. The chromatogram of the spiked sample (Figure 9b) showed that the height of the peak with a retention time of 4.7 min increased. Further experiments showed that the increase in height of the peak at 4.7 min was proportional to the amount of added reference penicillamine. Thus, penicillamine was verified to be present in acidic penicillin G solutions

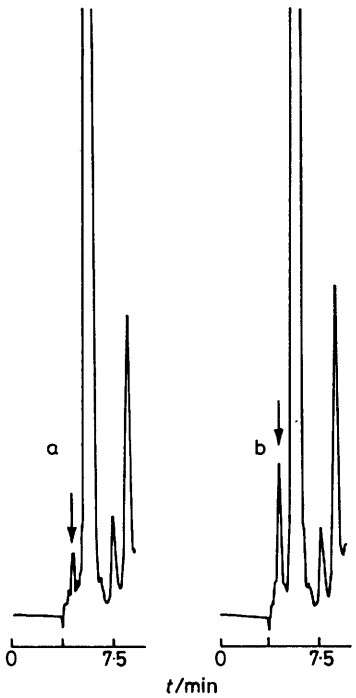


FIGURE 9 H.p.l.c. chromatograms of 0.05M-penicillin G aged for three days at pH 2.5, 37 °C showing presence of penicillamine. a, Unspiked; b, spiked with reference penicillamine; arrow indicates peak corresponding to pencillamine

during ageing by analysis with two different h.p.l.c. systems.

To further confirm the presence of penicillamine in acidic penicillin G solution during ageing, a d.p.p. method which is specific for the mercapto-group of penicillamine was used.[5,6] Neither benzylpenicillenic acid nor benzylpenamaldic acid interferes with this determination. Figure 10a shows that an acidic penicillin G solution which was aged for four days in 0.1M-phosphate buffer (pH 2.5) at 37 °C gave a peak potential value of −0.31 V, which is identical to the peak potential value for penicillamine.[6] The presence of penicillamine was further demonstrated when the aged solution was spiked with reference penicillamine. As seen in Figure 10b, the peak potential for the spiked sample was identical to the unspiked sample.

Conclusions.—Scheme 1 is supported both by kinetic analysis and additional analytical methods. Although it is true that a good fit of a mathematical model to experimental data does not prove the validity of the model, it is also true that a poor fit of a mathematical

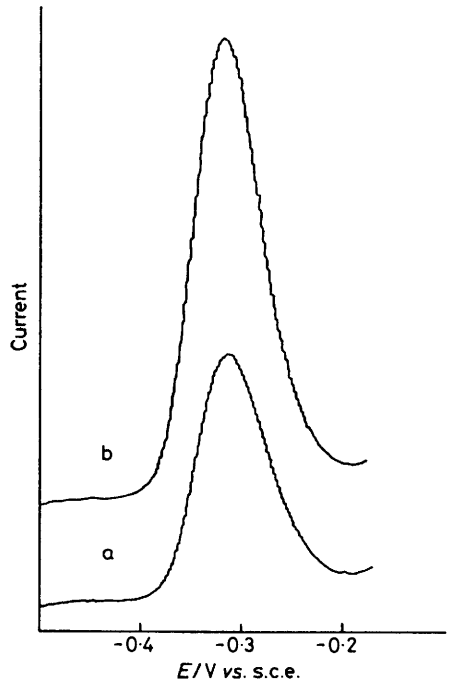


FIGURE 10 Differential pulse polarogram of 0.05M-penicillin G aged for four days at pH 2.5, 37 °C showing presence of penicillamine at −0.32 V. a, Unspiked; b, spiked with with reference pencillamine

model to experimental data raises questions about either the model or the data. The confirmation of the presence of penicillamine in aged penicillin G solutions by reversed-phase h.p.l.c. and by d.p.p. further justifies the preference for Scheme 1.

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