The Equilibrium between the Antibiotics Hetacillin and Ampicillin in Solution

By A. K. DURBIN and H. N. RYDON*

(*Department of Chemistry, University of Exeter, Exeter* EX4 4QD)

Summary It is shown that hetacillin and ampicillin are reversibly interconverted in aqueous acetone, by way of the Schiff's base derived from ampicillin and acetone, and rate and equilibrium constants for the overall reaction are evaluated.

HETACILLIN is the generic name for the antibiotic 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicillanic acid (I) obtained[1] by the action of acetone on ampicillin [6-(α-aminophenylacetamido)penicillanic acid; III]. It has been shown[2] that hetacillin is converted into ampicillin in aqueous solution but it is not known with certainty whether or not this reaction is reversible. We now report evidence which shows that the two antibiotics are in fact in equilibrium in aqueous solution and that the Schiff's base (II) is probably an intermediate in their interconversion.

The ^{1}H n.m.r. spectrum of a solution of the potassium salt of hetacillin in deuterium oxide changes with time, eventually, after 12—24 h, becoming that of a mixture of acetone and the potassium salts of hetacillin and ampicillin. This change can be used to follow the reaction, both in deuterium oxide and water, most conveniently by measuring the heights of the very sharp singlet peaks for the phenyl protons of hetacillin (τ 2·52) and ampicillin (τ 2·58).

Our experiments were carried out at 33°, at which temperature the first-order rate constant, k, for the disappearance of hetacillin, as its potassium salt, in water is $2{\cdot}32 \times 10^{-4}$ s^{-1} when the initial concentration is 12·5% and $2{\cdot}10 \times 10^{-4}$ s^{-1} when the initial concentration is 6·7%; the rate is not greatly affected by changing the solvent to deuterium oxide ($k = 1{\cdot}82 \times 10^{-4}$ s^{-1} for an initial concentration of 12·8%). As expected for a reversible reaction, the addition of acetone to the solution results in an increase in the proportion of potassium hetacillin in the equilibrium mixture and the same equilibrium mixture results from both potassium hetacillin and potassium ampicillin at any given final concentration of acetone. The composition of the equilibrium mixture was determined after 12 h (*ca.* 12 half-lives) at 33° for a range of antibiotic and acetone concentrations. The results are summarised in the Table in which are given both the uncorrected equilibrium constant, K, and the partially corrected constant, K', in which the concentrations of acetone and water are replaced by their activities, calculated from vapour pressure measurements on acetone–water mixtures.[3] The residual departure of K' from constancy is no doubt due to the variation of the activity coefficients of (I) and (III) over the range of solvent composition studied.

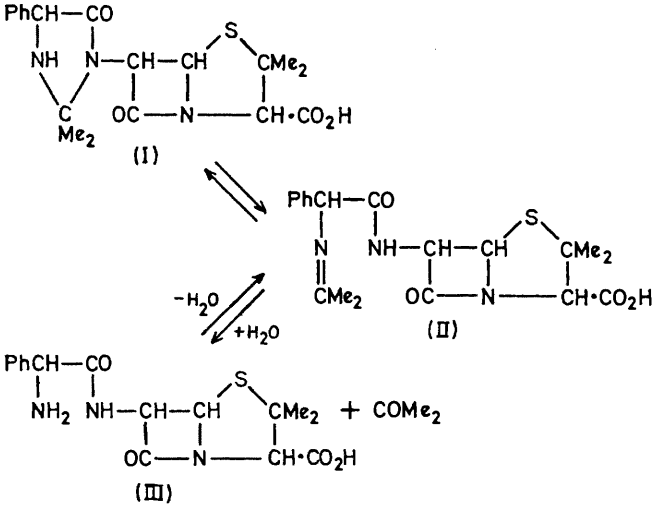
Evidence for the intervention of an intermediate in the reverse reaction, from ampicillin to hetacillin, was obtained by measuring the change with time of the optical rotation of a 0·1%-solution of ampicillin in pyridine in the presence of 100 equiv. of acetone; $[\alpha]_D$ first falls from an initial value of +180° to a minimum, +108°, reached after about 2 h at room temp., and then rises to a final value of +320°, reached in 40 h. Clearly the reaction involves an intermediate with a lower specific rotation than either ampicillin or hetacillin.

Evidence that this intermediate is the Schiff's base (II) was obtained by studying the change with time of the ^{1}H n.m.r. spectra of solutions of hetacillin and ampicillin in "anhydrous" hexadeuteriodimethyl sulphoxide, nearly all samples of which contain small amounts of water. When equilibrium is reached in such solutions, containing a deficiency of water, the complex ^{1}H n.m.r. spectrum shows the presence, not only of hetacillin, ampicillin, and acetone (singlet at τ 7·93), but also of another component, characterised by a singlet at τ 8·19. This fourth component is clearly the Schiff's base (II), the singlet at τ 8·19 being due to the ·N : CMe_2 protons; the intervention of an analogous Schiff's base has been established[4] in the conversion of oxytocin into a dimethyloxoimidazolidine by treatment with acetone.

Equilibrium constants in water at 33°

Initial concentrations, % Acetone	Antibiotic	Number of experiments	Mean value of 10^2K	$10^2K'$
0	6·7	1	0·76	0·68
0	12·5	1	1·28	0·61
0	12·8[a]	1	1·26	0·52
4·2—4·7	1·4—12·6	8[b]	2·06	0·81
7·8—9·8	1·4—12·8	8[b]	2·70	1·08
14·5—15·5	6·9—13·0	4[b]	4·18	1·75
20·0—21·5	7·0—13·2	4[b]	6·02	2·21

[a] Solvent deuterium oxide.
[b] Half starting from potassium hetacillin, half from potassium ampicillin.



Starting from hetacillin, the Schiff's base peak appears only after acetone has been formed in amount corresponding to the amount of water originally present; if there is an excess of water, the peak is only just detectable at equilibrium. Starting from ampicillin and acetone, the peak appears immediately and then decreases in size as equilibrium is approached. It may be concluded that the formation of the Schiff's base (II) is the slower of the two steps in the forward reaction from hetacillin to ampicillin, but the faster of the two in the back reaction. The same

equilibria are no doubt involved in the interconversion of the potassium salts of hetacillin and ampicillin in water or aqueous acetone since, under favourable conditions, the Schiff's base peak can just be detected at equilibrium in such systems.

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