The Reaction of Nocardicin A in Acid: Concurrent Hydrolysis of an Oxime and a β-Lactam

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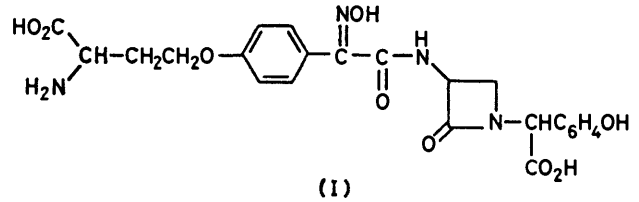
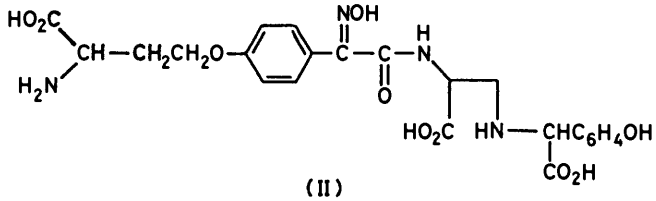
The mechanism of acid hydrolysis of the microbial antibiotic nocardicin A has been examined by a comparison of its kinetic dependence upon acid concentration with those of several β-lactams and oximes. It is concluded that, in moderately concentrated sulphuric acid, the faster reaction is β-lactam hydrolysis but that the spectral changes are due to hydrolysis of the oxime group.

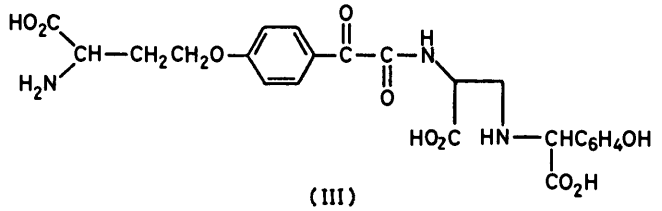
NOCARDICIN A (I) is an antibiotic of microbiological origin and is unusual in that it is a monocyclic β-lactam whereas penicillins and cephalosporins contain a fused β-lactam ring. Nocardicin A was first isolated by a group of Japanese workers [1] and the products of the alkaline and acid hydrolysis have been isolated and characterised.[2] They are (II) and (III). In the acid reaction



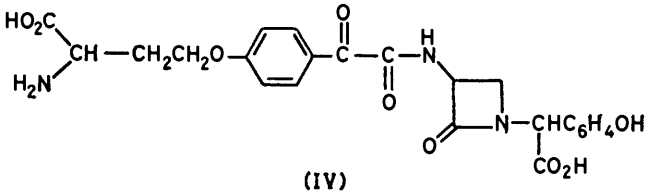
(I)



(II)



(III)



(IV)

both the oxime and the β-lactam functions are hydrolysed. The mechanism of the reaction could take one of three forms: (a) fast oxime hydrolysis to give (IV) and slow β-lactam hydrolysis, (b) fast β-lactam hydrolysis to give (II) and slow oxime hydrolysis, and (c) hydrolysis of the two groups occurring at comparable rates. In view of the functional groups around both the β-lactam ring and the oxime group it is difficult to predict, from data in the literature, the probable rates of hydrolysis of the two groups. We decided, therefore, to investigate the acid hydrolysis of nocardicin A by studies of the hydrolyses of model compounds.

RESULTS AND DISCUSSION

A solution of nocardicin A in 3M-hydrochloric acid exhibits spectral changes with the passage of time (see Figure 1). The principal changes are an increase in absorbance at 300 nm and a decrease at 270 nm. There is a tight isosbestic point at 287 nm. We cannot say, with certainty, which of the reactions listed above is responsible for the spectral changes. However, we used the change at 270 nm to measure the rate of acid hydrolysis. The product (III) was isolated after reaction

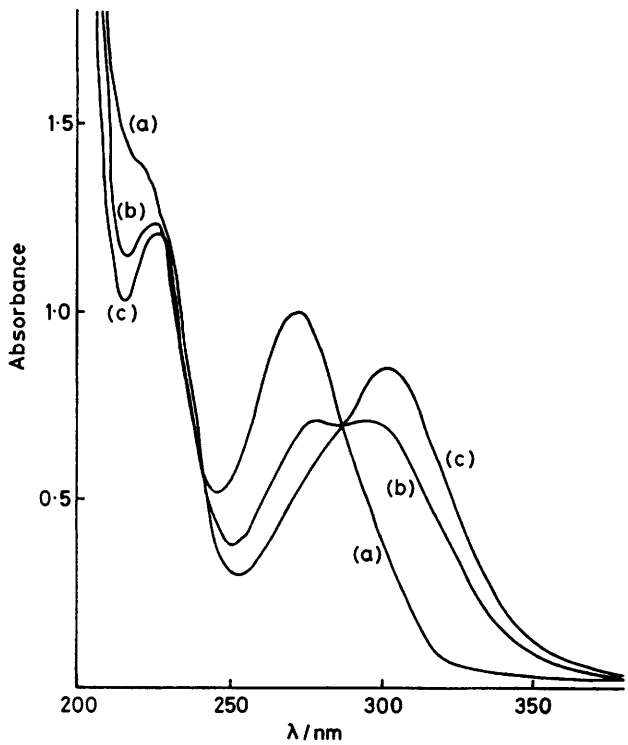


FIGURE 1 Spectral changes during the acid hydrolysis of nocardicin A: (a) initial; (b) after 3 h; (c) after 24 h

of nocardicin A in 3M-hydrochloric acid and so we associate the observed spectral changes with either oxime hydrolysis, or β-lactam hydrolysis, or both.

The hydrolyses of oximes and β-lactams which are model compounds for nocardicin A occur only in moderately concentrated acid and so, for the purposes of comparison, the reactions of nocardicin A in similar media were examined. Results for reaction in aqueous sulphuric acid are shown in Table 1. In spite of a fairly

TABLE 1

Hydrolysis of nocardicin A in aqueous sulphuric acid at 30 °C

% acid w/w	26.0	29.0	31.0	33.5	40.5	42.5	44.0	45.5
10^3 $k_{obs.}$/s^{-1}	0.40	0.55	0.64	0.84	1.70	2.05	2.25	2.55

[Nocardicin A]$_0$ 1.15 × 10^{-4}M

large change in acidity (as measured by any acidity function) there is only a small change in the value of $k_{obs.}$.

We next examined the hydrolyses of two β-lactams. The u.v. spectrum of *N*-phenylazetidin-2-one (V) in sulphuric acid has a maximum absorbance at 246 nm (ε_{246} 22 000) which decreased during the course of several hours and this we attribute to hydrolytic ring-opening. Good first-order plots were obtained for the variation of absorbance at 246 nm with time. The results are displayed in Table 2. Similar data for *N*-4-nitrophenylazetidin-2-one (VI), for which $\lambda_{max.}$ is 327 nm (ε_{327} 14 400), are also displayed in Table 2. Further data for the

TABLE 2

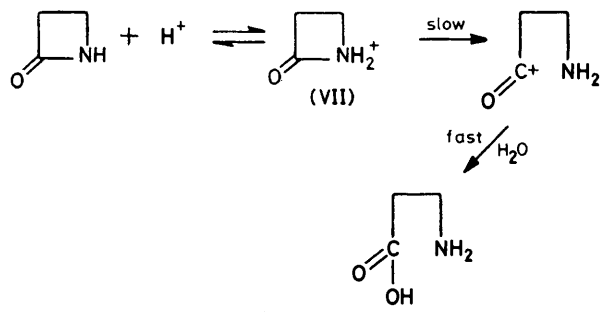
Hydrolysis of *N*-phenylazetidin-2-one (V) and *N*-4-nitrophenylazetidin-2-one (VI) in aqueous sulphuric acid at 30 °C

	10^5 $k_{obs.}$/s^{-1}	
% acid w/w	(V)	(VI)
27.0		2.51
30.0		3.52
32.0	2.35	5.00
34.5	3.60	7.52
36.5	4.45	11.5
37.5	6.20	17.0
40.5	9.75	24.0
42.5	12.5	32.5
44.0	17.0	54.5
45.5	20.5	63.0

[*N*-phenylazetidin-2-one]$_0$ 6.8 × 10^{-3}M
[*N*-4-nitrophenylazetidin-2-one]$_0$ 1.04 × 10^{-4}M

hydrolysis of the unsubstituted β-lactam, azetidin-2-one, are available from the work of Yates *et al.*[3]

We can at this stage comment, briefly, upon the mechanism of β-lactam hydrolysis in acid. Yates *et al.*[3] have presented good evidence that β-lactams, in contrast to other lactams, react by an *A*-1 mechanism (Scheme 1). Although predominant protonation occurs on the carbonyl oxygen [4] there is some *N*-protonation [5] and it is this species (VII) which undergoes ring opening. The data of Table 2 support this mechanism for the hydrolyses of (V) and (VI) as there is no evidence of a maximum in the rate profile, such as that found by Yates *et al.*[3]



SCHEME 1

for lactams of larger ring size. The mechanism of reaction for these compounds is a tetrahedral intermediate type (A_0T2), where the activity of water plays an important role. The presence of a phenyl group attached to the nitrogen lowers its basicity and so it is right that azetidin-2-one reacts more rapidly than (V). The 4-nitro-groups should have the same effect but the opposite was observed: it enhances the rate of reaction. However, because of the lower basicity of 4-nitroaniline the 4-nitro-group attached to the phenyl group of (V) generates a better leaving group and this compensates for the smaller extent of protonation. A semi-logarithmic rate profile for the hydrolysis of the three β-lactams gives three parallel curves (see Figure 2) and suggests that all three react by the same mechanism, *i.e.* an *A*-1 mechanism. It seems reasonable, therefore, to assume that hydrolysis of the β-lactam ring of nocardicin A occurs by the same mechanism but we do not know, yet, if it is the slow step.

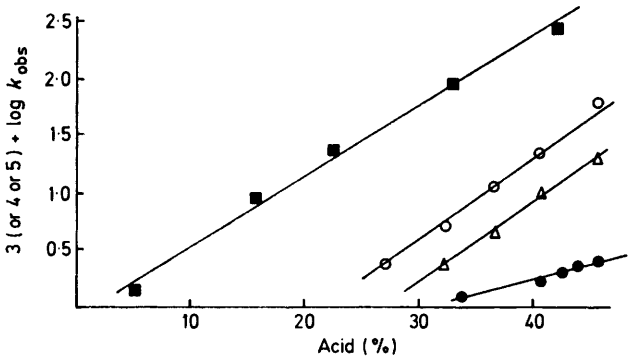
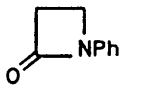


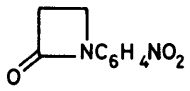
FIGURE 2 Hydrolysis in sulphuric acid: ■ 4 + log $k_{obs.}$ for azetidin-2-one; ○ 5 + log $k_{obs.}$ for *N*-4-nitrophenylazetin-2-one; △ 5 + log $k_{obs.}$ for *N*-phenylazetidin-2-one; ● 3 + log $k_{obs.}$ for nocardicin A

We now consider the acid hydrolysis of oximes as models for the other part of the nocardicin A molecule undergoing reaction in acid solution. The oxime function of nocardicin is distinctive in that it is attached to an amide group. This may affect its reactivity and we found no data in the literature on this point.

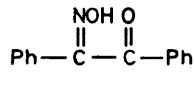
The hydrolysis of acetophenone oxime in moderately concentrated sulphuric acid has been considered in detail by Gregory and Moodie [6] and they propose the mechanism shown in Scheme 2. There is independent evidence that protonation of an oxime occurs



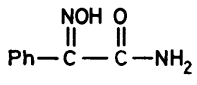
(V)



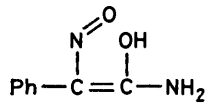
(VI)



(VIII)

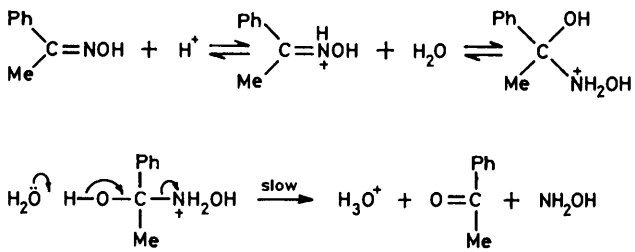


(IX)



(X)

on nitrogen.[7] Because the slow step of Scheme 2 involves water the rate of reaction in moderately concentrated acid is affected, not only by the acidity, but also by the activity of water. The consequence of this is that from 10.1% acid upwards the rate of reaction decreases with increasing acid concentration. Similar results were obtained in a study of the hydrolysis of cyclohexanone and cyclopentanone oximes in moderately concentrated acid.[8] As the data in Table 1 show, this is not the case with nocardicin A and we might conclude that the process giving rise to the spectral changes in the hydrolysis of nocardicin A is not oxime hydrolysis. However, we need to examine a better model for the oxime function of nocardicin A and for this we chose first the mono-oxime of benzil, (VIII).



SCHEME 2

Reaction of (VIII) in 45.5% w/w aqueous sulphuric acid over 4 h produced essentially no spectral changes. No kinetic studies were, therefore, possible and so we cannot ascertain the effect of a neighbouring carbonyl group upon the hydrolysis of an oxime. We had more success with the oxime of benzoylformamide, (IX). In 45.5% w/w aqueous sulphuric acid there is a gradual decrease in absorbance at 250 nm and an increase at 260 nm. However the isosbestic point was not tight and the continuing increase at 260 nm we thought might be due to hydrolysis of benzoylformamide, the product of oxime hydrolysis, to benzoylformic acid. This was confirmed by an examination of the spectral changes occurring on addition of an authentic sample of benzoylformamide to aqueous sulphuric acid. There is the same gradual increase of absorbance at 260 nm and values of $k_{obs.}$ for amide hydrolysis as a function of acid concentration are displayed in Table 3. The final spectrum is the same as that obtained from the acid hydrolysis of benzoylformamide mono-oxime (IX) and so the decrease in absorbance at 250 nm during the reaction of (IX) must be due to hydrolysis of the oxime function. This spectral change occurs more rapidly than hydrolysis of benzoylformamide and so, although the isosbestic point is not tight, it is possible to obtain reasonable kinetic data for oxime hydrolysis. These are displayed in Table 3. The reaction is *ca.* 30 times faster than hydrolysis of benzoylformamide.

TABLE 3

Hydrolyses of benzoylformamide (XI) and benzoylformamide mono-oxime (IX) in aqueous sulphuric acid at 30 °C

% acid w/w	(XI) $10^5 k_{obs.}/s^{-1}$	(IX) $10^3 k_{obs.}/s^{-1}$
26.0		0.45
29.0		0.60
31.0		0.69
33.5		0.85
40.5	2.65	1.45
42.5	3.51	1.65
44.0	4.00	1.90
45.5	4.62	2.00

[Benzoylformamide]$_0$ 10^{-4}M
[Benzoylformamide mono-oxime]$_0$ 10^{-4}M

The hydrolysis of this oxime does not show the same type of dependence on acid concentration as does acetophenone mono-oxime.[6] With the latter, in the same acid concentration range, there is a decrease in the value of $k_{obs.}$. This difference must be due to the proximity of the amide group in (IX). In moderately concentrated acid acetophenone oxime is essentially completely protonated and so for its hydrolysis the value of $k_{obs.}$ is fixed by the water activity. As (IX) is a much weaker base, for reasons which will be discussed below, the extent of protonation increases with an increase of acidity but this effect is counteracted by a decrease in the concentration of the other species involved in formation of the tetrahedral intermediate, *viz.* water (Scheme 2). Inspection of the data in Table 3 shows that there is only a small increase in the value of $k_{obs.}$ as the acid concentration is increased.

What is significant in our understanding of the overall acid hydrolysis of nocardicin A is that plots of $k_{obs.}$ for its hydrolysis and for that of benzoylformamide oxime are approximately coincident (Figure 3) and so it is reasonable to suggest that the spectral changes observed during acid hydrolysis of nocardicin A are due to oxime hydrolysis. We have made no assumptions about the most appropriate acidity function for an analysis of the kinetic data for oxime hydrolysis. The use by Yates *etal* .[3] of percentage composition as a non-committal measure of acidity is an admirable compromise in view of the proliferation of acidity functions. If the same data for nocardicin A are plotted alongside those for β-lactam hydrolysis (Figure 2) the curve is not parallel to the other three and this is evidence that the observed spectral changes during nocardicin A hydrolysis are not due to hydrolysis of the β-lactam ring. A semilogarithmic plot

was necessary in this instance because of the large changes in the value of $k_{obs.}$.

If we compare the rates of hydrolysis of azetidin-2-one and benzoylformamide mono-oxime it is seen that in the range 40.5—45.5% acid the former is the faster reaction. If opening of the β-lactam ring in nocardicin A occurs by the same mechanism as that of azetidin-2-one, then it is reasonable to propose that, in nocardicin hydrolysis,

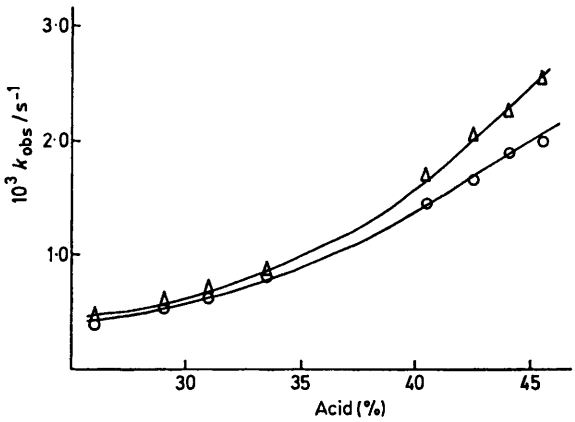


FIGURE 3 Hydrolysis in sulphuric acid: ○ benzoylformamide mono-oxime, △ nocardicin A

oxime hydrolysis is the slower step. The spectral changes are, therefore, due to the hydrolysis of (II). As hydrolyses of the two functional groups depend differently upon the acid concentration, at lower acidity this situation is probably reversed and β-lactam hydrolysis is the slower process. This is unlikely to be reflected in the nature of the spectral changes observed. Neither reaction occurs at high pH, such as that in biological systems, and so the acid hydrolysis of nocardicin A is not important in its use as an antibiotic.

The rate data of Gregory and Moodie [6] suggest that acetophenone oxime is a fairly strong base and Vinnik and Zarakhani [8] report a pK_a value of 3.3 for cyclohexanone oxime. However, it is clear that benzoylformamide oxime is not completely protonated in moderately concentrated acid and must, therefore, be a much weaker base. The same effect was observed by Ellefsen and Gordon,[9] who reported a pK_a value of −3.16 for diacetyl mono-oxime. The change in the basicity of the oxime nitrogen must be due to the neighbouring group. The same effect is shown in the hydrolysis of nocardicin A in dilute acid. In this pH range, where the activity of water is constant, acetophenone mono-oxime is completely protonated and the value of $k_{obs.}$ is independent of pH. This is not the case with nocardicin A as the data in Table 4 show. These reactions are very slow (half-life typically 15 h) but there is a linear relationship between hydrogen ion concentration and $k_{obs.}$, with a second-order rate constant of 1.7×10^{-5} l mol^{-1} s^{-1}. This linearity would be exhibited only by a weak base. We suggest that the low basicity is due to hydrogen bonding between the oxime group and the neighbouring carbonyl. In its extreme form this would lead to formation of a nitrone (X) and nitrones are known to be weak bases.

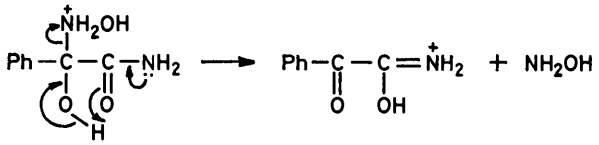
TABLE 4

Hydrolysis of nocardicin A in aqueous buffer at 37 °C

pH	1.75	1.15	1.05	0.75
$10^5 k_{obs.}/s^{-1}$	0.80	1.23	1.70	2.80

$[\text{Nocardicin A}]_0$ 1.15×10^{-4}M

Although the presence of an amide group lowers the basicity of benzoylformamide oxime it still hydrolyses more rapidly than acetophenone mono-oxime. It could be that the slow step in oxime hydrolysis, proton removal from the tetrahedral intermediate, is effected in this case not by another water molecule (as in Scheme 2) but by the neighbouring amide group (Scheme 3). Thus the



SCHEME 3

amide group in benzoylformamide mono-oxime and nocardicin A may exercise two functions, that of reducing the basicity of oxime but enhancing the reactivity of the hydrated form.

EXPERIMENTAL

Materials—The sodium salt of nocardicin A was generously supplied by the Fujisawa Pharmaceutical Co., Osaka, and *N*-phenylazetidin-2-one and *N*-4-nitrophenylazetidin-2-one were prepared as described by Butler *et al.*[10] Benzil oxime [11] and benzoylformic acid [12] were prepared by literature methods. The latter was converted into benzoylformyl chloride by a modification of the method of Kharasch and Brown.[13] The acid (15 g) was dissolved in dry oxalyl chloride (3 ml) and the solution refluxed on a steam-bath for 6 h in the absence of moisture. The excess of oxalyl chloride was removed by evaporation and toluene (50 ml) added to the residue. After filtration the toluene was removed by evaporation and the residue distilled; b.p. 65 °C at 0.5 mmHg. This material (11 g) was added dropwise to cooled concentrated aqueous ammonia (150 ml). The yellow precipitate was filtered off, washed with dilute aqueous ammonia, and dried to give benzoylformamide (yield 4.6 g, 48%), m.p. 125 °C (lit.,[14] 129 °C). This was converted into the oxime by the usual procedure [11] and recrystallised from ethanol (yield 0.8 g, 60%), m.p. 172 °C (Found: C, 58.7; H, 5.0; N, 16.7. $C_8H_8N_2O_2$ requires C, 58.5; H, 4.9; N, 17.1%).

Buffer solutions were prepared from AnalaR hydrochloric acid and the ionic strength maintained at 0.2M by addition of KCl. The concentrations of sulphuric acid were determined by density measurements.

Kinetics.—Rate constants were determined by the standard procedure by the use of a Unicam SP8-100 spectrophotometer. The rate constants were calculated by the method of Swinbourne.[15]

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