Studies on the Hydrolysis of Clavulanic Acid

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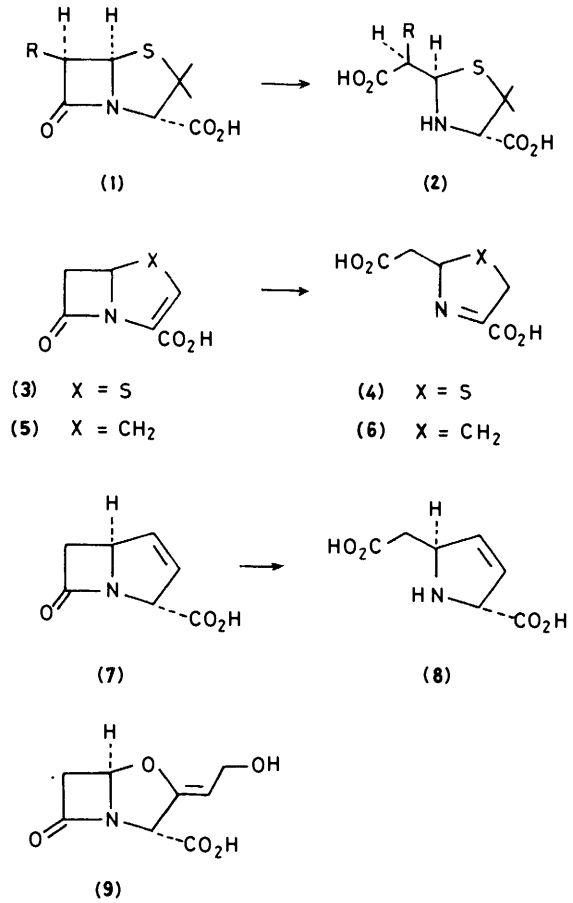
1-Amino-4-hydroxybutan-2-one (**14**) has been identified as one of the major products from the hydrolysis of clavulanic acid in acidic, alkaline, or neutral solution. In alkaline or neutral solution, the amino ketone (**14**) is converted into other products, including two pyrazines, 2,5-bis(2-hydroxyethyl)pyrazine and 3-ethyl-2,5-bis(2-hydroxyethyl)pyrazine. A rationale for the formation of these products is discussed.

It is well known that the penicillins (**1**) are hydrolysed by alkali and by β-lactamase enzymes to give the well characterised penicilloic acids (**2**).[1] Also, in the penem and carbapenem series, it has been shown[2] that the three synthetic representatives (**3**), (**5**), and (**7**) are hydrolysed by alkali to give, respectively, the three dicarboxylic acids (**4**), (**6**), and (**8**), which can be isolated and characterised as their bis(*p*-nitrobenzyl) esters. A common feature of the above reactions is that the products retain the five-membered ring of the original 4,5-fused bicyclic system. It is found, however, that this generalisation does not follow for the 4,5-fused ring system of the naturally occurring β-lactamase inhibitor clavulanic acid (**9**). Although, as will be discussed below, the reasons for this are fairly obvious, the isolation and characterisation of the hydrolysis products from this clinically useful natural product (**9**) have never been reported. The aim of the work to be described in this paper was to identify and characterise the major products resulting from the hydrolysis of acid (**9**).

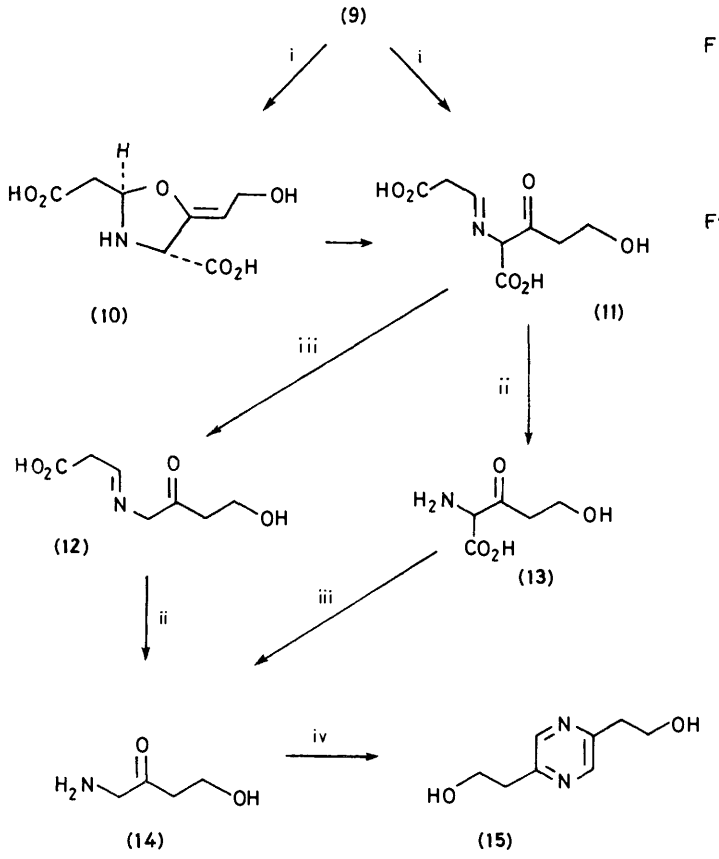
A postulated series of reactions that might follow on hydrolysis of the β-lactam group in the acid (**9**) is shown in Scheme 1. Simple hydrolysis of the β-lactam ring would give the oxazolidine (**10**), which would be expected to tautomerise to the imine (**11**). Another possibility is that hydrolysis of the β-lactam moiety with concomitant opening of the oxazolidine ring would lead directly to the imine (**11**). Thus, hydrolysis of the β-lactam group of clavulanic acid (**9**), unlike that of the penams, penems, and carbapenems, is expected to be accompanied by destruction of the five-membered ring. Under hydrolytic conditions, the intermediate (**11**) could undergo two further reactions, namely decarboxylation of the β-keto acid and hydrolysis of the imine, to give, eventually, the amino ketone (**14**). Depending on the order of these latter reactions, the decarboxylated imine (**12**) or the α-amino β-keto acid (**13**) might be intermediates in this process. It follows that, on the basis of this scheme, the expected products from the hydrolysis of clavulanic acid (**9**) would be the amino ketone (**14**), acetaldehyde, and two molecules of carbon dioxide. Under acidic conditions, the amino ketone might be stable; however, in neutral or basic solution this compound would be expected to undergo a self-condensation reaction leading to the pyrazine (**15**).

The above scheme is in accordance with previous studies on the hydrolysis of clavulanic acid. Haginaka[3] and his co-workers found that in phosphate buffer at pH 7.83 clavulanic acid was hydrolysed to products which showed $\lambda_{max.}$ at 276 nm in the u.v. spectra, whereas hydrolyses at pH 5.00 and 3.24 yielded products that were transparent at 276 nm. We would expect the pyrazine (**15**) to show u.v. absorption with $\lambda_{max.}$ around 276 nm.

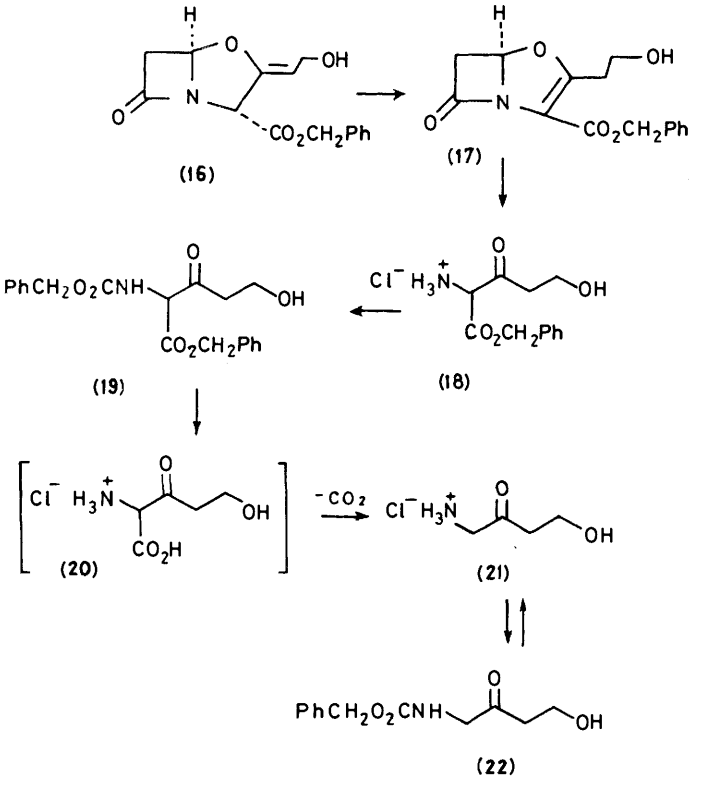
In our own work, initial studies on the hydrolysis of clavulanic acid with alkali or acid yielded complex mixtures of products when examined by t.l.c. and n.m.r. spectroscopy. We therefore decided to prepare the amino ketone (**14**) [as its hydrochloride (**21**)] by the indirect route outlined in Scheme 2. Benzyl clavulanate (**16**) was isomerised to the clavem (**17**) using triethylamine in dichloromethane.[4] The clavem (**17**) was then hydrolysed using dilute HCl (1.2 equiv.) in 1,4-dioxane over 0.5 h.† Removing the solvent from the hydrolysis mixture gave a product which, from its i.r. and n.m.r. spectra, appeared to be the amino ester hydrochloride (**18**). In order to characterise this product and to facilitate purification, compound (**18**) was *N*-acylated using benzyl chloroformate to give the crystalline derivative (**19**) in an overall yield of 44% from benzyl clavulanate (**16**). The derivative (**19**) was deprotected by



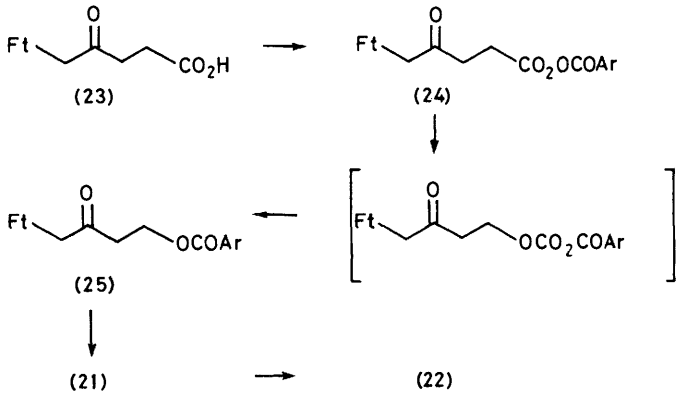
† The hydrolysis of benzyl clavulanate (**16**) under these conditions was slower and gave a complex mixture of products when examined by t.l.c. and n.m.r. spectroscopy.



Scheme 1. *Reagents:* i, H_2O; ii, H_2O, $-CO_2$, $-CH_3CHO$; iii $-CO_2$; iv, (**14**), $-2H_2O$, $-2[H]$



Scheme 2.



Scheme 3. Ft = phthalimido, Ar = 3-chlorophenyl

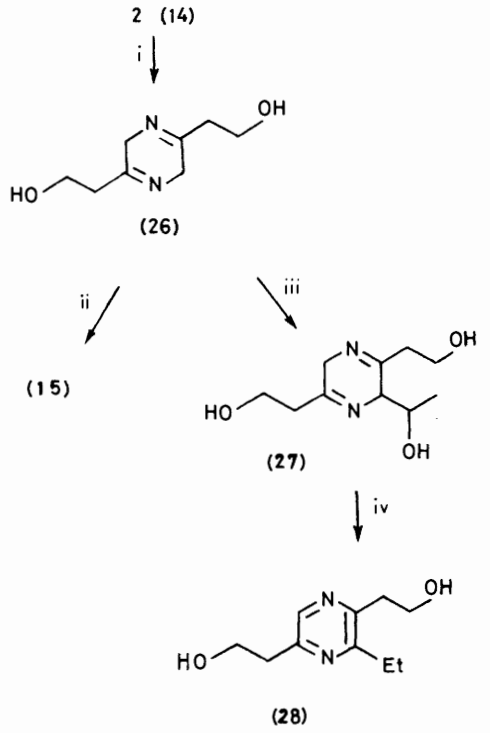
catalytic hydrogenation in the presence of HCl (1.2 equiv.) to give, in almost quantitative yield, the desired amino ketone hydrochloride (**21**); presumably, the amino acid hydrochloride (**20**) is an intermediate in this process. The hydrochloride (**21**) was obtained as a gum which was characterised by its spectral properties. Further characterisation of compound (**21**) was provided by converting it into its crystalline *N*-benzyloxycarbonyl derivative (**22**), which was obtained in 94% yield. The amino ketone hydrochloride (**21**) could be regenerated from its derivative (**22**) by hydrogenation in the presence of HCl. The hydrochloride (**21**) could also be obtained by hydrogenation of the amino ester hydrochloride (**18**) in water.

When compound (**19**) was hydrogenated in neutral water–tetrahydrofuran, the only product to be isolated was the crystalline pyrazine (**15**) in 83% yield. As already discussed above, the formation of this pyrazine is an expected reaction of the amino ketone (**14**) under neutral conditions. In water, compound (**15**) showed u.v. absorption with $\lambda_{max.}$ 274 nm, which is in close agreement with the $\lambda_{max.}$ reported by Haginaka[3] for the products from the hydrolysis of clavulanic acid at pH 7.83.

We have also prepared the amino ketone hydrochloride (**21**) from 5-phthalimidolevulinic acid[5] as shown in Scheme 3. The acid (**23**) was converted into the diacyl peroxide (**24**) using *m*-chloroperoxybenzoic acid and dicyclohexylcarbodi-imide in dichloromethane (46% yield). Heating the diacyl peroxide in toluene caused it to rearrange with loss of carbon dioxide to give the ester (**25**) (21%). Compound (**25**), which is a protected derivative of the desired amino ketone, was then hydrolysed in refluxing 6M-HCl to give the crude amino ketone hydrochloride (**21**). This product was purified and characterised by way of its *N*-benzyloxycarbonyl derivative (**22**) [21% overall yield from compound (**25**)], which proved to be identical with compound (**22**) obtained from benzyl clavulanate (Scheme 2).

With the pyrazine (**15**) and the amino ketone hydrochloride (**21**) available as reference compounds, we re-examined the hydrolysis of clavulanic acid in acidic, basic, and neutral solutions. In dilute HCl (0.2M; 1.25 equiv.), clavulanic acid (**9**) was hydrolysed during 2 h to give a complex mixture of products when examined by t.l.c. and n.m.r. spectroscopy. From t.l.c., the amino ketone hydrochloride (**21**) appeared to be a major component in this mixture of products, and signals due to compound (**21**) were clearly discernible in the n.m.r. spectrum. As expected, the pyrazine (**15**) did not appear to be present. Acylation of the crude hydrolysis product using benzyl chloroformate, followed by chromatography, gave the derivative (**22**) [15% overall yield from the acid (**9**)], thus providing a more rigorous proof for the presence of compound (**21**). The other products detected in the hydrolysis mixture have not been identified.

When potassium clavulanate was hydrolysed in dilute alkali



Scheme 4. *Reagents:* i, $-2H_2O$; ii, $-2[H]$; iii, CH_3CHO; iv, $-H_2O$

(0.02M-KOH; 1 equiv.), the amino ketone (**14**) was detected within a few minutes by t.l.c. In the initial stages of the reaction this was the only product detected, but as the hydrolysis proceeded the picture became more complex with other products, including the pyrazine (**15**), appearing in the hydrolysis mixture. Repeating the hydrolysis with water–tetrahydrofuran as solvent and trapping the amino ketone (**14**) with benzyl chloroformate gave the derivative (**22**) (25% yield from potassium clavulanate). Thus, in both the acid and alkaline hydrolysis of clavulanic acid (**9**), the amino ketone (**14**) is produced as one of the major hydrolysis products.

As with the hydrolysis in alkali, the hydrolysis of potassium clavulanate in neutral solution (0.042M in water) appeared to give initially the amino ketone (**14**), followed by a more complex mixture of products as the reaction was allowed to proceed. The reaction was repeated on a preparative scale, and after 3 days at 40 °C the hydrolysis mixture was subjected to preparative high performance liquid chromatography. In this way, samples of two of the major products were obtained pure: one of these was the previously described pyrazine (**15**) (10.5%), and the other was assigned the pyrazine structure (**28**) (10%) on the basis of its spectral properties.§ Possible routes to these two compounds are outlined in Scheme 4. As discussed earlier, self-condensation of the amino ketone (**14**) would give the intermediate (**26**), which on aerial oxidation would give the pyrazine (**15**). An alternative fate for the intermediate (**26**) would involve its reaction with acetaldehyde to give a further intermediate (**27**), which could aromatise by loss of water to give the second pyrazine (**28**). On the basis of this scheme, the isolation of the pyrazine (**28**) provides some evidence for acetaldehyde being a hydrolysis product of clavulanic acid.

§ While our paper was being prepared, a brief note (J. Haginaka, H. Yasuda, T. Uno, and T. Nakagawa, *Chem. Pharm. Bull.*, 1983, **31**, 1812) appeared describing the isolation of the pyrazine (**28**) from the degradation of potassium clavulanate in aqueous alkali (pH 9.21). The n.m.r. and mass spectra quoted in this note for compound (**28**) are in accord with our own data for this compound.

Some support for the above proposals was provided by the observation that the amino ketone hydrochloride (**21**) in water containing an equivalent of acetaldehyde gave rise to the pyrazines (**15**) and (**28**) when the solution was treated with an equivalent of sodium hydrogen carbonate. The pyrazine (**28**) (8%) was isolated from this reaction by chromatography. In an analogous experiment in which the acetaldehyde was omitted pyrazine (**15**) was again produced, but there was no sign of the 3-ethylpyrazine (**28**).

In conclusion, we can say that the amino ketone hydrochloride (**21**) is one of the major products from the hydrolysis of clavulanic acid (**9**) in dilute HCl. In alkali or neutral solution, the amino ketone (**14**) is again produced as one of the major hydrolysis products from compound (**9**), but as the hydrolysis is allowed to proceed this gives rise to other products including the pyrazines (**15**) and (**28**). The identification of the pyrazine (**28**) suggests that acetaldehyde is also produced during the hydrolysis reaction. These observations are all consistent with the reactions outlined in Scheme 1 being involved in the hydrolysis of clavulanic acid. Finally, the above findings are of interest not only from the purely chemical point of view, but also because the reactions and intermediates suggested in Scheme 1 could be implicated in certain biological processes that involve hydrolysis of the β-lactam moiety of the acid (**9**).

Experimental

M.p.s. were determined using a Kofler hot-stage apparatus. Except where stated otherwise, i.r. spectra were recorded for solutions in chloroform and ^1H n.m.r. spectra were recorded at 90 MHz for solutions in $CDCl_3$ with $SiMe_4$ as internal standard. U.v. spectra were recorded for solutions in water. Mass spectra were determined using a V.G. Micromass 70-70F instrument. Merck silica gel 60 was used for column chromatography with ethyl acetate–light petroleum as eluant. Light petroleum refers to the fraction boiling in the range 60–80 °C. Ether refers to diethyl ether. Tetrahydrofuran (THF) was purified by distillation just before being used. Solutions were dried using sodium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evaporator with the bath temperature below 30 °C.

Thin layer chromatography (t.l.c.) was performed on Merck silica gel 60 with the following solvent systems: (A) ethyl acetate–methanol–water–acetic acid (60:30:9:1); (B) chloroform–methanol–acetic acid (50:50:1); and (C) propan-2-ol–ethanol–water–90% formic acid (94:48:56:5).

Benzyl 2-Amino-5-hydroxy-3-oxopentanoate Hydrochloride (**18**).—Benzyl clavulanate (**16**) (3.0 g) was dissolved in dry dichloromethane (60 ml) and triethylamine (1.0 g) was added to the stirred solution. The mixture was stirred for 0.5 h and was then diluted with dichloromethane (40 ml) and washed with water (30 ml) containing 1M-HCl (10 ml) and water (30 ml). The solution was dried, diluted with 1,4-dioxane (75 ml), and the dichloromethane was removed. The resulting pale yellow solution of the clavem (**17**)[4] was stirred while 1M-HCl (12 ml) was added, and stirring was then continued for 0.5 h. The dioxane was removed and the resulting residue was dissolved in water (50 ml). The solution was washed with ethyl acetate (30 ml) and ether (2 × 20 ml), and was then filtered, de-gassed, and freeze-dried to give the benzyl ester (**18**) as a pale yellow foam (2.2 g); $\nu_{max.}$(KBr) 3 400, 1 750, and 1 725 cm^{-1}; $\delta(D_2O)$ 2.92 (2 H, t, *J* 5.5 Hz), 3.68 (2 H, t, *J* 5.5 Hz), 5.24 (2 H, s), and 7.34 (5 H, s).

Benzyl 2-Benzyloxycarbonylamino-5-hydroxy-3-oxopentanoate (**19**).—The benzyl ester (**18**) (2.2 g) was dissolved in a mixture of water (40 ml) and THF (40 ml). The solution was stirred and ice-cooled while a solution of benzyl chloroformate

(1.7 g) in THF (10 ml) was added dropwise during 5 min, and at the same time sodium hydrogen carbonate (1.6 g) was added in small portions. Stirring and ice-cooling were continued for 10 min, and then the cooling bath was removed and stirring was continued for a further 30 min. The mixture was diluted with ethyl acetate (150 ml) and washed with water, dilute HCl, and saturated brine. The solution was dried, the solvent was removed, and the residue was chromatographed to give the *ester* (**19**) as a pale yellow gum (1.7 g), which crystallised from ether–pentane as colourless needles, m.p. 63—64 °C; $\nu_{max.}$ 3 420, 3 250, 1 750sh, 1 720, 1 500, and 700 cm^{-1}; δ 2.27br (1 H, s, exchanges with D_2O), 2.82 (2 H, t *J* 5.5 Hz), 3.80 (2 H, t, *J* 5.5 Hz), 5.08 and 5.19 (5 H, two s overlapping m), 6.05br (1 H, d, *J* 6 Hz, exchanges with D_2O), 7.31 (10 H, s), and 12.30 (*ca.* 0.1 H, s, enolic OH); *m/z* 371 (M^+, 0.1), 299 (0.5), 254 (0.5), 253 (0.6), 238 (0.7), 220 (0.5), 210 (0.5), 208 (0.7), 181 (3), 164 (2.5), 148 (4), 108 (2), 107 (4), 91 (100), 73 (10), and 65 (8) (Found: C, 64.25; H, 5.65; N, 3.7%; M^+, 371.136. $C_{20}H_{21}NO_6$ requires C, 64.65; H, 5.7; N, 3.75%; *M*, 371.135).

Hydrogenation of the Ester (**19**) *in the Presence of* HCl.—The ester (**19**) (1.4 g) was dissolved in a mixture of THF (30 ml), water (5 ml), and 1M-HCl (4.5 ml) and the solution was shaken with 10% palladium–charcoal (350 mg) under hydrogen at 1 atm for 20 min. The catalyst was removed by filtration and was washed with water. The solvent was removed from the filtrate and the resulting residue was dissolved in ethanol (50 ml) and filtered. The solvent was removed, the resulting residue was dissolved in ethanol (30 ml), and again the solvent was removed. The residue was dried *in vacuo* (phosphorus pentaoxide) to give 1-*amino*-4-*hydroxybutan*-2-*one hydrochloride* (**21**) as a pale yellow gum (540 mg) which appeared to be homogeneous as judged by t.l.c. [solvent (A), R_F 0.05; solvent (B), R_F 0.1; solvent (C), R_F 0.4]; $\nu_{max.}$(KBr) 3 390, 2 980, and 1 725 cm^{-1}; δ(D_2O) 2.78 (2 H, t, *J* 6 Hz), 3.82 (2 H, t, *J* 6 Hz), and 4.05 (2 H, s); δ_C (62.9 MHz; D_2O) 43.03 (C-3), 48.43 (C-1), 56.93 (C-4), and 204.79 p.p.m. (C-2); the positive-ion fast atom bombardment mass spectrum* for a suspension of the hydrochloride (**21**) in glycerol showed *inter alia m/z* 104 ($[M - Cl]^+$, 100%).

1-*Benzyloxycarbonylamino*-4-*hydroxybutan*-2-*one* (**22**).—The amino ketone hydrochloride (**21**) (520 mg) was converted into the ketone (**22**) by reaction with benzyl chloroformate (720 mg) under the conditions described earlier for the conversion of (**18**) into (**19**). The ketone (**22**) was obtained as colourless flakes (830 mg), m.p. 57—58 °C (ethyl acetate–light petroleum); $\nu_{max.}$ 3 420, 1 715, and 1 510 cm^{-1}; δ 2.25br (1 H, s, exchanges with D_2O), 2.64 (2 H, t, *J* 5.5 Hz), 3.87 (2 H, t, *J* 5.5 Hz), 4.08 (2 H, d, *J* 5 Hz), 5.10 (2 H, s), 5.45br (1 H, t, *J* 5 Hz, exchanges with D_2O), and 7.32 (5 H, s) (Found: C, 60.85; H, 6.55; N, 6.0. $C_{12}H_{15}NO_4$ requires C, 60.75; H, 6.35; N, 5.9%).

Hydrogenation of the Ketone (**22**).—The ketone (**22**) (240 mg) was hydrogenated in a manner similar to that described for the ester (**19**) in the presence of HCl. The amino ketone hydrochloride (**21**) was obtained as a yellow gum (140 mg) with properties as previously described.

Hydrogenation of the Benzyl Ester (**18**).—The benzyl ester (**18**) (600 mg) in water (15 ml) was shaken with 10% palladium–charcoal (200 mg) under hydrogen at 1 atm for 30 min. The catalyst was removed by filtration and was washed with water. The solvent was removed from the filtrate to give the amino ketone hydrochloride (**21**) as an almost colourless gum (310 mg) with properties as previously described.

* This spectrum was obtained using a VG-ZAB mass spectrometer with high-energy xenon atoms as the fast atoms.

Hydrogenation of the Ester (**19**) *in Neutral THF*.—The ester (**19**) (370 mg) in a mixture of THF (15 ml) and water (4 ml) was shaken with 10% palladium–charcoal (90 mg) under hydrogen at 1 atm for 25 min. The catalyst was removed by filtration and was washed with THF. The solvent was removed from the filtrate to yield a brown gum which was chromatographed (using 1:9 ethanol–ethyl acetate as eluant) to give 2,5-*bis*(2-*hydroxyethyl*)*pyrazine* (**15**) as pale brown crystals (70 mg). A sample of the product was sublimed at 80—90 °C/0.5 mmHg to give colourless prisms, m.p. 90.5—92 °C; $\lambda_{max.}$ 204 (ε 9 700), 274 (7 600), and 294infl. nm (2 180); $\nu_{max.}$(KBr) 3 310, 1 500, 1 490, and 1 475 cm^{-1}; δ(CD_3SOCD_3) 2.83 (4 H, t, *J* 6.5 Hz), 3.72 (4 H, q, *J* 6.5 Hz, becoming t on D_2O exchange), 4.60 (2 H, t, *J* 6.5 Hz, exchanges with D_2O), and 8.40 (2 H, s); *m/z* 168 (M^+, 25), 167 (13), 151 (46), 138 (37), 125 (27), 120 (18), and 107 (100) (Found: C, 57.3; H, 7.25; N, 16.55%; M^+, 168.090. $C_8H_{12}N_2O_2$ requires C, 57.15; H, 7.2; N, 16.65%; *M*, 168.090).

3-*Chlorobenzoyl* 5-*Phthalimidolevulinoyl Peroxide* (**24**).—5-Phthalimidolevulinic acid[5] (**23**) (2.19 g) was dissolved in dry dichloromethane (50 ml), and 3-chloroperoxybenzoic acid (1.45 g) and dicyclohexylcarbodi-imide (1.73 g) were added to the solution, which was then stirred at room temperature for 40 h. The mixture was filtered, the solvent was removed from the filtrate, and the resulting residue was chromatographed. The *peroxide* (**24**) was thus obtained as colourless crystals (1.48 g), m.p. 135—136 °C (ethyl acetate–light petroleum); $\nu_{max.}$ 1 805, 1 775, 1 735sh, and 1 720 cm^{-1}; δ 2.7—3.1 (4 H, m), 4.55 (2 H, s), and 7.3—8.0 (8 H, m) (Found: C, 57.5; H, 3.5; N, 3.5. $C_{20}H_{14}ClNO_7$ requires C, 57.75; H, 3.4; N, 3.35%).

4-(3-*Chlorobenzoyloxy*)-1-*phthalimidobutan*-2-*one* (**25**).—The diacyl peroxide (**24**) (0.65 g) was dissolved in dry toluene (50 ml). The solution was heated by an oil bath with the temperature being gradually increased from 60 to 110 °C during 6 h. The mixture was then heated at 100 °C (bath temperature) for a further 6 h. The solution was cooled, the solvent was removed, and the resulting gum was chromatographed. The *ester* (**25**) was thus obtained as colourless crystals (0.12 g), m.p. 111—112 °C (ethyl acetate–light petroleum); $\nu_{max.}$ 1 780 and 1 720 cm^{-1}; δ 3.00 (2 H, t, *J* 6 Hz), 4.55 (2 H, s), 4.64 (2 H, t, *J* 6 Hz), and 7.25—8.05 (8 H, m) (Found: C, 61.9; H, 4.1; N, 3.9. $C_{19}H_{14}ClNO_5$ requires C, 61.4; H, 3.8; N, 3.75%).

Hydrolysis of the Ester (**25**).—The ester (**25**) (120 mg) was suspended in 6M-HCl (3 ml) and the mixture was stirred and refluxed for 5.5 h. The mixture was cooled, diluted with water (10 ml), and filtered. The filtrate was washed with ethyl acetate (10 ml) and ether (2 × 10 ml), and then the solvent was removed to give the crude amino ketone hydrochloride (**21**) as a pale brown gum (22 mg); δ(D_2O) *inter alia* 2.79 (2 H, t, *J* 6 Hz), 3.83 (2 H, t, *J* 6 Hz), and 4.07 (2 H, s).

The above product (22 mg) was converted into its *N*-benzyloxycarbonyl derivative (**22**) using the method previously described. The ketone (**22**) was obtained as colourless crystals (16 mg), m.p. 56—57 °C, with i.r. and n.m.r. spectra as previously described.

Hydrolysis of Clavulanic Acid (**9**) *in Dilute* HCl.—Clavulanic acid (**9**) (0.8 g) was dissolved in water (20 ml) and 1M-HCl (5 ml) was added to the stirred solution. After 2 h, t.l.c. showed that all the clavulanic acid had been consumed, and the solvent was removed from the hydrolysis mixture to give a brown gum (0.65 g). The amino ketone hydrochloride (**21**) was detected in this crude product by t.l.c. and n.m.r. spectroscopy: δ(D_2O) *inter alia* 2.75 (t, *J* 6 Hz), 3.79 (part overlapped t, *J* 6 Hz), and 4.01 (s).

The total crude product was brought into reaction with

benzyl chloroformate (720 mg), under the conditions described earlier, to give the ketone (**22**) as colourless crystals (140 mg), with i.r. and n.m.r. spectra as previously described.

Hydrolysis of Potassium Clavulanate with Potassium Hydroxide.—Potassium clavulanate (60 mg) was dissolved in water (10 ml) and 1M-potassium hydroxide (0.25 ml) was added to the solution. The hydrolysis was followed by t.l.c. [solvent systems (A) and (B)]. After 15 min, the amino ketone (**14**) could be detected in the solution by t.l.c. [solvent (A), R_F 0.05; (B), R_F 0.1]. After 30 min, almost all the potassium clavulanate had been consumed. After 45 min, together with the amino ketone (**14**), the pyrazine (**15**) could be detected in the solution [solvent (A), R_F 0.45; (B), R_F 0.55].

The hydrolysis was repeated on a larger scale. Potassium clavulanate (240 mg) in water (20 ml)–THF (20 ml) was treated with 1M-potassium hydroxide (1 ml). After 30 min, benzyl chloroformate (0.2 ml, 240 mg) was added to the solution which was then stirred for 45 min. The mixture was diluted with ethyl acetate (150 ml) and washed with water, dilute HCl, and saturated aqueous sodium hydrogen carbonate. The solution was dried, the solvent was removed, and the residue was chromatographed to give the ketone (**22**) as colourless crystals (60 mg), with i.r. and n.m.r. spectra as previously described.

Hydrolysis of Potassium Clavulanate in Water.—Potassium clavulanate (10 g) in de-ionised water (1 l) was stirred at 40 °C for 72 h. The mixture, which had pH 6.9, was filtered and the filtrate was freeze-dried. The resulting residue was chromatographed on a Waters Associates Prep LC/500 equipped with a Prep Pak 500/C_{18} cartridge, eluting with 1:9 acetonitrile–water at a rate of 100 ml min^{-1} and using a refractive index detector. The fractions collected between 3.5 and 5 min were combined and re-chromatographed as above to give the pyrazine (**15**) as colourless crystals (376 mg). Also, the fractions collected between 7 and 10 min were combined and re-chromatographed to give 3-*ethyl*-2,5-*bis*(2-*hydroxyethyl*)*pyrazine* (**28**) as colourless crystals (420 mg). The pyrazine (**15**) had spectral properties as previously described plus: δ_C (20 MHz; D_2O) 37.51 (CH_2CH_2OH), 61.40 (CH_2OH), 144.83 (C-3 and C-6), and 153.10 p.p.m. (C-2 and C-5). The pyrazine (**28**) was obtained as colourless crystals, m.p. 60.5—62 °C; $\lambda_{max.}$ 210 (ε 9 700), 278 (8 700), and 296 infl. nm (3 950); $\nu_{max.}$(KBr) 3 410, 3 110, 1 530, 1 483, and 1 455 cm^{-1}; δ(CD_3SOCD_3) 1.21 (3 H, t, *J* 8 Hz), 2.68—3.01 (6 H, m), 3.76 (4 H, q, *J* 6.5 Hz, becoming t on D_2O exchange), 4.62 (2 H, t, *J* 6.5 Hz, exchanges with D_2O), and 8.26 (1 H, s); δ_C (20 MHz; D_2O), 13.75 (CH_3), 27.56 (CH_2 of ethyl), 36.28 (CH_2CH_2OH), 37.45 (CH_2CH_2OH), 61.25 (CH_2OH), 61.43 (CH_2OH), 141.76 (C-6), 150.60 (C-2, C-3, or C-5), 152.25 (C-2, C-3, or C-5), and 157.92 p.p.m. (C-2, C-3, or C-5); *m/z* 196 (M^+, 70%), 179 (43), 166 (38), and 135 (100) (Found: C, 60.95; H, 8.35; N, 14.3%; M^+, 196.121. $C_{10}H_{16}N_2O_2$ requires C, 61.2; H, 8.2; N, 14.3%; *M*, 196.121).

Reaction of the Amino Ketone Hydrochloride (**21**) *with Acetaldehyde.*—The amino ketone hydrochloride (**21**) (280 mg) in water (40 ml) was treated with acetaldehyde (90 mg) and sodium hydrogen carbonate (170 mg). The solution was kept for 24 h, after which time t.l.c. showed a complex mixture of products including the 3-ethylpyrazine (**28**) [solvent (A), R_F 0.58] and the pyrazine (**15**) [solvent (A), R_F 0.45]. The solvent was removed from the reaction mixture and the resulting residue was chromatographed (using 1:9 ethanol–ethyl acetate as eluant) to give the pyrazine (**28**) as a pale yellow gum (16 mg) with an n.m.r. spectrum as previously described.

In a control experiment, the hydrochloride (**21**) (280 mg) in water (40 ml) was treated with sodium hydrogen carbonate (170 mg) and the solution was kept for 24 h. T.l.c. of the reaction mixture showed a mixture of products, including the pyrazine (**15**); it was clear from t.l.c. that the 3-ethylpyrazine (**28**) was not present.

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