Preparation of the 7-Oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene System and the Reversible Cleavage of its Oxazoline Ring

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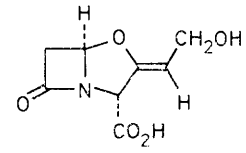
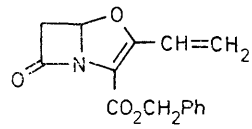
Summary Treatment of the derivative (**5**) of clavulanic acid with triethylamine leads to the endocyclic double bond isomer (**6**) and thence to the salt (**10**) which is a powerful inhibitor of β-lactamases; cleavage of the oxazoline ring of (**6**) with triethylamine or with pyridine gives the novel zwitterions (**7**) and (**9**) which are readily recyclised to (**6**) by thermolysis.

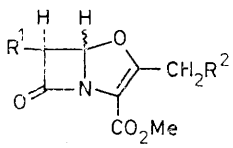
REPORTS from these[1] and other laboratories[2] have described the isolation of a novel, bicyclic, β-lactamase inhibitor, clavulanic acid (**1**). In a search for related compounds possessing similar biological properties, we were interested in a simple, general method for preparing isomers in which the exocyclic double bond of clavulanic acid and its derivatives has been moved into the 5-membered ring and into conjugation with the carboxylate group. This 7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene ring system, which has been described recently in the form of the benzyl ester[3] (**2**) of the diene derived from clavulanic acid and as the semi-synthetic methyl esters (**3a**)[4] and (**3b**)[5], is an oxygen analogue of the newly synthesised[6] pen-2-em system (**4**), a structure combining important features of both penicillins and cephalosporins.



(1)

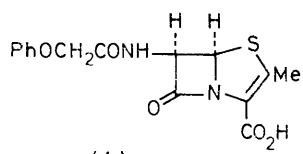


(2)



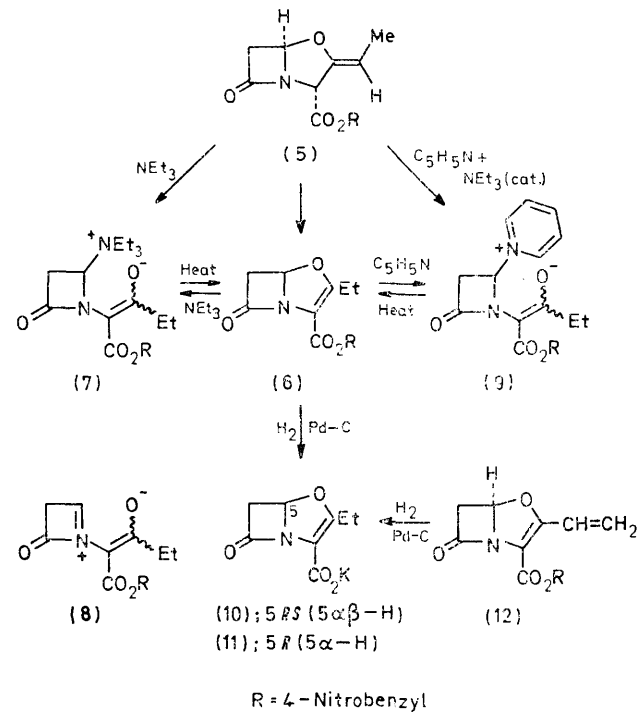
(3)

a; $R^1 = Ph_3CNH$, $R^2 = H$
b; $R^1 = H$, $R^2 = Me$



(4)

Our attempts to isomerise derivatives of clavulanic acid resulted not only in a convenient preparation of the required compounds (*e.g.*, **10**) but also in the discovery of some unexpected chemistry of this ring system. Thus, treatment of 4-nitrobenzyl (2*R*,5*R*)-*Z*-3-ethylidene-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate (**5**), prepared from clavulanic acid by hydrogenolysis[7] and esterification, with triethylamine (2 equiv.) in ethyl acetate at ambient temperature for 24 h resulted in the deposition of a very polar (t.l.c.) and highly crystalline product which was not the expected isomer (**6**) but was identified as the betaine, 1-(4-nitrobenzyloxycarbonyl)-1-(2-oxo-4-triethylammonioazetidin-1-yl)but-1-en-2-olate† (**7**; 62%); m.p. 116—118 °C (decomp.); λ_{max} (pH 6 buffer) 273·5 nm (ε 29,800); ν_{max} (Nujol) 1770 (β-lactam) and 1660 cm^{-1} (ester). However, the isolated betaine rapidly dissolved when heated under reflux in ethyl acetate and within 2 min had been cleanly converted into the desired 4-nitrobenzyl 3-ethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene-3-carboxylate (**6**) which was isolated as a crystalline solid (78%); m.p. 121—122 °C, ν_{max} (Nujol) 1806 (β-lactam) and 1698 cm^{-1} (ester). Treatment of this αβ-unsaturated ester under the same conditions as used in the conversion of (**5**) into (**7**) resulted in the regeneration of the betaine (**7**; 87%).



R = 4-Nitrobenzyl

Although the shift of the double bond could precede or follow the opening of the 5-membered ring in the formation of the betaine (**7**) from the ester (**5**), we suggest that the isomerisation occurs as the first step to give the αβ-unsaturated ester (**6**) which is then in reversible equilibrium with the betaine (**7**). Owing to its low solubility in ethyl acetate at ambient temperature, the betaine is removed from solution by crystallisation and the equilibrium is displaced in its favour. However, if chloroform is used as solvent, crystallisation does not occur and after 7 h an equilibrium mixture of the αβ-unsaturated ester (**6**) and the betaine (**7**) has been established in solution. This equilibrium can be approached from either side, as well as from (**5**) itself. Thus, when treated respectively with 4, 4, and

† The proposed structures of all new compounds were supported by ^1H n.m.r. spectroscopy.

3 equiv. of triethylamine at ambient temperature, equimolar solutions of (**5**), (**6**), and (**7**) in deuteriochloroform became indistinguishable (n.m.r., i.r., and t.l.c. examination) after 7 h and were seen by n.m.r. spectroscopy to contain only (**6**) and (**7**) in the ratio *ca.* **1 : 1**. These results explain the recently reported[5] failure to deconjugate the double bond of the methyl ester (**3b**) under similar conditions.

Neither the betaine (**7**) isolated from reaction of (**5**) in ethyl acetate nor the αβ-unsaturated ester (**6**) derived from it by heating showed any detectable rotation at the D-line, indicating both to be racemic. However, reactions of (**5**) in chloroform solution gave an initial rise in rotation of *ca.* 20% over the first hour showing that at least one of the initially formed products retains a chiral centre originating from C-5 in the optically active starting ester. As the reaction proceeded racemisation of the mixture occurred and the rotation fell to near zero after 24 h. The observed racemisation may result from the intermediacy of the achiral azetidinium salt (**8**) in the equilibrium established between the αβ-unsaturated ester (**6**) and the betaine (**7**).

TABLE
Concentrations of the test compounds μg ml^{-1} required to halve the rates of hydrolyses of the substrates by cell-free β-lactamase enzymes

Enzyme	Type[a]	Substrate	Clavulanic acid[b] (**1**)	Potassium salt (**10**)
PC1	G +ve Penase	AMP[c]	8·2	0·021
P99	G −ve I	CER[d]	>25	0·01
RP1	G −ve III	CER	0·18	0·88
K1	G −ve IV	CER	0·15	0·44

[a] M. H. Richmond and R. B. Sykes, *Adv. Microbial Physiol.*, **1973**, **9**, 31. [b] Tested as the lithium salt. [c] AMP = Ampicillin. [d] CER = Cephaloridine.

A further example of the ready and reversible opening of the oxazoline ring of (**6**) was provided by its reaction with pyridine in ethyl acetate for 3 h to afford a precipitate of 1-(4-nitrobenzyloxycarbonyl)-1-(2-oxo-4-pyridinioazetidin-1-yl)but-1-en-2-olate (**9**; 51%); λ_{max} (pH 6 buffer) 272 nm (ε 26,200); ν_{max} (Nujol) 1781 cm^{-1} (β-lactam). Brief reflux of a suspension of the pyridinium zwitterion (**9**) in ethyl acetate again smoothly regenerated the αβ-unsaturated ester (**6**).

Under the same conditions as used to prepare (**9**) from (**6**), the ester (**5**) failed to react, presumably because pyridine is too weak a base to effect the initial isomerisation of the double bond to give the αβ-unsaturated ester (**6**). However, addition of a catalytic amount of triethylamine to a solution of (**5**) in pyridine resulted in its conversion into the zwitterion (**9**).

Deprotection of the αβ-unsaturated ester (**6**) by hydrogenation over 10% palladium on carbon gave the corresponding racemic acid which was isolated as the potassium salt (**10**); $[\alpha]_D$ 0° ± 1° (*c* 1·02, H_2O); λ_{max} (H_2O) 260·5 nm (ε 5,800); ν_{max} (Nujol) 1772 (β-lactam) and 1570 cm^{-1} (carboxylate).

Apart from its lack of optical activity, this salt was the same as the potassium salt (**11**), $[\alpha]_D$ + 84° (*c* 1·34, H_2O), which was obtained by hydrogenation of the optically active diene[8] (**12**) in carefully purified (basic alumina column) ethyl acetate. Failure to purify the solvent in this way resulted in the isolation of the racemic salt (**10**), again showing the optical instability of the 7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene system.

The Table shows that, like clavulanic acid (**1**), the potassium salt (**10**) is a potent inhibitor of cell-free β-lactamase enzymes derived from both Gram-positive and Gram-negative organisms and indeed shows markedly better activity than clavulanic acid against the Staphyloccocal penicillinase PC1 and the P99 enzyme from *Enterobacter cloacae*. These results are in contrast to the reportedly weak activity of the methyl ester (**3a**).[4]

(*Received, 3rd March* 1978; *Com.* 224.)

[1] Glaxo Laboratories Ltd., German OLS No. 2,604,697.
[2] T. T. Howarth, A. G. Brown, and T. J. King, *J.C.S. Chem. Comm.*, 1976, 266.
[3] D. F. Corbett, T. T. Howarth, and I. Stirling, *J.C.S. Chem. Comm.*, 1977, 808.
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[6] R. B. Woodward, 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' ed. J. Elks, Special Publication No. 28, The Chemical Society, London, 1977, p. 167.
[7] A. G. Brown, J. Goodacre, J. B. Harbridge, T. T. Howarth, R. J. Ponsford, I. Stirling, and T. J. King, ref. 6, p. 295.
[8] P. C. Cherry, G. I. Gregory, C. E. Newall, P. Ward, and N. S. Watson, preceding communication.