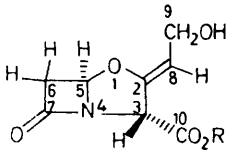
Clavulanic Acid, a Novel β-Lactam isolated from *Streptomyces clavuligerus*; *X*-Ray Crystal Structure Analysis

By T. TREFOR HOWARTH and ALLAN G. BROWN*
(*Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey* RH3 7AJ)
and TREVOR J. KING
(*Department of Chemistry, The University, Nottingham* NG7 2RD)

Summary The structure of clavulanic acid, a β-lactamase inhibitor isolated from *Streptomyces clavuligerus*, has been shown by spectroscopic methods and *X*-ray analysis to be a novel fused β-lactam.

STREPTOMYCES CLAVULIGERUS has been shown to produce penicillin N, 7-(5-amino-5-carboxyvaleramido)-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid, 7-(5-amino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylic acid,[1] and deacetoxycephalosporin C.[2]

During a programme of screening for novel β-lactamase inhibitors it was discovered that the above organism produced a new type of β-lactam antibiotic, clavulanic acid,[3] *i.e.* *Z*-(2*R*,5*R*)-3-(β-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3,2,0]heptane-2-carboxylic acid. In this communication we present evidence in support of structure (**1a**) for clavulanic acid.



(1)

a; R = H
b; R = Me
c; R = *p*-$NO_2C_6H_4CH_2$
d; R = *p*-$BrC_6H_4CH_2$

Methylation of sodium clavulanate with methyl iodide in dimethylformamide gave methyl clavulanate (**1b**)† as an oil $[\alpha]_D^{22}$ + 38·0°, *M*+ 213·0635 ($C_8H_{11}NO_5$); ν_{max} (film) 1800 (β-lactam CO), 1750 (ester CO), and 1695 cm^{-1} (O–C=C–); λ_{max} no absorption >210 nm. The n.m.r. spectrum contained an ABX system [δ 3·05 (1H, dd, *J* 17·5 and 0·8 Hz, 6β-H), 3·54 (1H, dd, *J* 17·5 and 2·8 Hz, 6α-H), and 5·72 (1H, dd, *J* 2·8 and 0·8 Hz, 5-H)] corresponding to the β-lactam protons; the downfield shift of the C-5 proton and the smaller *cis* (2·8 Hz) and *trans* (0·8 Hz) coupling constants observed in (**1**) compared with penicillanic acid derivatives[4] illustrates the effect of replacing sulphur by oxygen in these bicyclic β-lactam systems.[5] The C-9 methylene protons appeared as part of an AA'X system at δ 4·24; the adjacent C-8 proton (the X part of the AA'X system) was further allylically coupled to the C-3 proton and appeared at δ 4·93 (ddd, *J* 7·2, 6·5, and 1·2 Hz). The C-3 proton was observed as a broad doublet at δ 5·07 owing to allylic coupling with the C-8 proton (*J* 1·2 Hz) and long-range coupling with the C-9 protons. The ^{13}C n.m.r. spectra showed nine carbon resonances at δ 174 (s, C-7 or C-10), 167 (s, C-10 or C-7), 152 (s, C-2), 100 (d, C-8), 87 (d, C-5), 60 (d, C-3), 57 (t, C-9), 53 (q, OCH_3), and 46 p.p.m. (t, C-6). The base peak in the electron impact mass spectrum was at *m/e* 196, corresponding to $(M - OH)^+$. On the basis of the above evidence it was possible to propose the gross structure represented by (**1**) or the corresponding *E*-isomer.

Structure (**1a**) for clavulanic acid and the relative stereochemistry were unambiguously elucidated by *X*-ray analysis of the *p*-nitrobenzyl ester (**1c**),† m.p. 117·5—118 °C, prepared from sodium clavulanate and *p*-nitrobenzyl bromide in dimethylformamide. *Crystal data:* $C_{15}H_{14}N_2O_7$, *M* 334, monoclinic, space group $P2_1$, a = 19·528(6), b = 7·795(3), c = 5·025(1) Å, β = 95·85(3)°. The structure was solved using MULTAN.[6] During refinement it became apparent that the primary alcohol group was disordered, the oxygen occupying two preferred positions. The final *R* value, with hydrogen atoms included, was 5·5%.

The crystal structure of *p*-bromobenzyl clavulanate (**1d**),† m.p. 103—104 °C, was determined by the heavy atom method. *Crystal data:* $C_{15}H_{14}BrNO_5$, *M* 368·2, monoclinic, space group $P2_1$, a = 13·155(6), b = 4·763(2), c = 12·670(5) Å, β = 95·22(2)°.

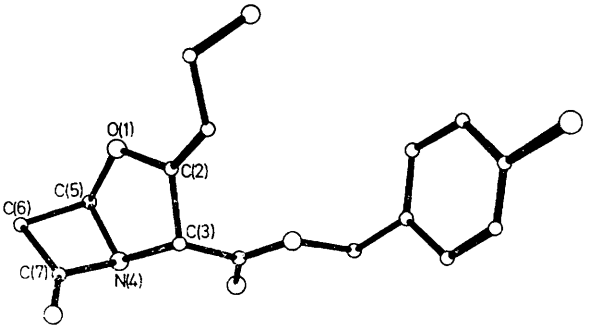


FIGURE. Structure of *p*-bromobenzyl clavulanate

All reflections were measured with their Friedel pairs and analysis of the results of including the anomalous dispersion of bromine in the calculations by both the statistical method of Hamilton[7] and by comparison of the intensities of suitable Friedel pairs established the absolute configuration as shown in the Figure.

Clavulanic acid (**1a**) is unique in that it is the first reported naturally occurring fused β-lactam containing oxygen instead of sulphur and it does not possess the acylamino-side chain present in penicillins and cephalosporins.

Clavulanic acid (as its sodium salt) is a potent irreversible inhibitor of various β-lactamases. When combined *in vitro* with ampicillin at a level of 5 μg/ml it reduced the minimum inhibitory concentration of ampicillin from >1000 μg/ml to <1·0 μg/ml against a β-lactamase producing strain of *Staphylococcus aureus*.

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† All new compounds gave spectral and analytical data in accord with ascribed structures.

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[5] The smaller coupling constants may also reflect distortion of the azetidinone ring in this system, *cf.* R. J. Stoodley and N. S. Watson, *J.C.S. Perkin I*, 1975, 883 and references cited therein.
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