Structures of Three Novel β-Lactams isolated from *Streptomyces clavuligerus*

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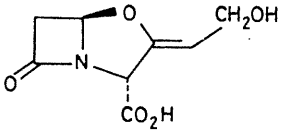
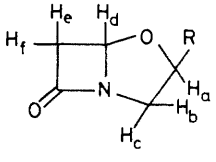
Summary The structures of three novel fused β-lactams isolated from culture fluids after growth of *Streptomyces clavuligerus* were shown by spectroscopic and degradative methods to be related to that of clavulanic acid.

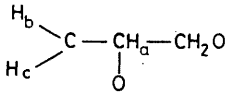
A RECENT report[1] described the structure of clavulanic acid (**1**), a β-lactam antibiotic with a novel bicyclic ring system, isolated from *Streptomyces clavuligerus*. While working with fermentations of this organism, we have discovered three more β-lactam metabolites[2] and in this communication present evidence in support of structures (**2**), (**3**), and (**4**) for these compounds. We propose the name, clavam, for the 7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane nucleus by analogy with the term, cepham, used in naming the cephalosporin antibiotics.[3]



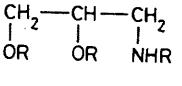
(1)



(2), R = CH_2OH
(3), R = CH_2OCOH
(4), R = CO_2H
(5), R = CO_2Na
(6), R = CO_2Me
(7), R = CO_2CHPh_2



(8)



(9), R = H
(10), R = COPh

2-Hydroxymethylclavam (**2**) was isolated as a colourless oil, $C_6H_9NO_3$, $[\alpha]_D^{23}$ − 166°, ν_{max} ($CHBr_3$) 3560 (OH) and 1770 cm^{-1} (β-lactam CO). The formyl ester (**3**) was isolated as a colourless oil, $C_7H_9NO_4$, ν_{max} ($CHBr_3$) 1784 (β-lactam CO) and 1730 cm^{-1} (ester CO). Clavam-2-carboxylic acid (**4**) was a solvent extractable acid and was difficult to separate from clavulanic acid (**1**). The material was characterised as the methyl ester (**6**), a colourless oil, $C_7H_9NO_4$, ν_{max} ($CHBr_3$) 1785 (β-lactam CO) and 1754 cm^{-1} (ester CO) and also as the diphenylmethyl ester (**7**) recrystallised from toluene as white needles, m.p. 146—147 °C; $[\alpha]_D^{23}$ − 109°; ν_{max} (Nujol) 1780 (β-lactam CO) and 1750 cm^{-1} (ester CO). The parent acid (**4**) was recovered from (**7**) by catalytic hydrogenation. The acid was isolated as the amorphous sodium, lithium, or magnesium salt; analytical samples were not obtained.

The structures of these metabolites follow from their ^1H n.m.r. spectra (Table). In addition to the six protons assigned (H_a to H_f), (**2**) shows the protons of a primary alcohol group and (**3**) those of the formyl ester analogue, whereas methyl and diphenylmethyl derivatives of (**4**) show only the signals of the ester functions.

The three signals, H_d, H_e, and H_f, were assigned as the β-lactam protons since the observed chemical shifts and coupling constants ($J_{d,e}$ 2·5—3, $J_{d,f}$ *ca.* 0, and $J_{e,f}$ 16—17 Hz) show good agreement with the values obtained for clavulanic acid derivatives.[1] The signals H_a, H_b, and H_c formed an ABX system and the coupling constants ($J_{a,b}$ 7—8, $J_{b,c}$ 11—12, and $J_{a,c}$ 4·5—6 Hz) indicated a $-CH_2CH-$ group. The part structure (**8**) was inferred from the spectra of (**2**) and (**3**), in which the presence of the oxygen atom follows from the chemical shift of the H_a proton.

The part structure (**8**) was confirmed by treatment of (**2**) with dilute acid, giving 3-aminopropan-1,2-diol (**9**), characterised as its tribenzoyl derivative (**10**), m.p. 134—135 °C, $[\alpha]_D^{23}$ + 29·3°. The i.r. and ^1H n.m.r. spectra of (10) were identical to those of authentic racemic material.[4]

TABLE. ^{1}H N.m.r. data of clavams (2), (3), and (5)—(7).

Assignment	Chemical shifts (δ/p.p.m.)				
	(2) [$(CD_3)_2CO$]	(3) ($CDCl_3$)	(5) (D_2O)	(6) ($CDCl_3$)	(7) ($CDCl_3$)
H_a	4·42	4·57	4·74	4·84	4·90
H_b	3·86	4·00	4·19	4·12	4·15
H_c	2·91	2·80	3·04	3·10	3·06
H_d	5·32	5·32	5·52	5·48	5·47
H_e	3·28	3·30	3·44	3·31	3·29
H_f	2·71	2·81	2·92	2·86	2·85
$-CH_2-$	3·68	4·24	—	—	—
–OH	4·23	—	—	—	—
–CHO	—	8·08	—	—	—
$-CO_2Me$	—	—	—	3·76	—
$-CO_2CHPh_2$	—	—	—	—	7·31, 6·92

The stereochemistry of these compounds has not been determined. These compounds exhibit antibiotic activity of a different type from clavulanic acid. Whereas clavulanic acid is an inhibitor of β-lactamase and of bacterial growth, 2-hydroxymethylclavam, 2-formyloxymethylclavam, and methyl clavam-2-carboxylate exhibit activity against a number of species of fungi, particularly fungal plant pathogens.

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[1] T. T. Howarth, A. G. Brown, and T. J. King, *J.C.S. Chem. Comm.*, 1976, 266.
[2] Ger. P. 2725690.
[3] R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, 1962, **84**, 3400.
[4] R. B. Bruce, L. Turnbull, J. Newman, and J. Pitts, *J. Medicin. Chem.*, 1966, **9**, 286.