Bioorganic & Medicinal Chemistry Letters, Vol.3, No.11, pp. 2255-2258, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

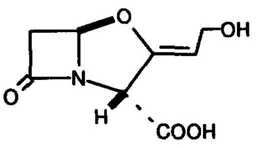
Synthesis and β-Lactamase Inhibitory Activity of 6-Hydroxyethyl Thiaclavulanic Acid.

Walter Cabri, Angelo Bedeschi,* Ettore Perrone, Giuseppina Visentin, Daniela Jabes and
Giovanni Franceschi.
Farmitalia Carlo Erba, via Giovanni XXIII, 23 20014-Nerviano (MI), Italy.

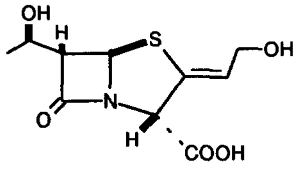
(*Received* 1 *April* 1993)

Abstract- 6-Hydroxyethyl thiaclavulanic acid, possessing the "natural" configuration both at C3 and at the double bond, was synthesized in only two steps from the penem nucleus *via* a palladium catalysed vinyl epoxide reductive rearrangement. The β-lactamase inhibitory activity is reported.

The β-lactamase inhibitory activity of clavulanic acid **1** has produced a large effort in the searching for new non classical β-lactams possessing enhanced properties.[1] In this context the formal synthesis of a thiaclavulanic acid analog has previously been reported by us.[2] Notably, even if some thiaclavulanic acid derivatives have been described,[3] the "natural" (Z)-hydroxymethyl derivative has never been synthesized. Intrigued by the β-lactamase inhibitory activity recently observed in some 7-hydroxyethyl cephems[4] and 6-hydroxymethyl oxapenems,[5] we became interested in exploring the activity of the 6-hydroxyethyl thiaclavulanic acid **2**.

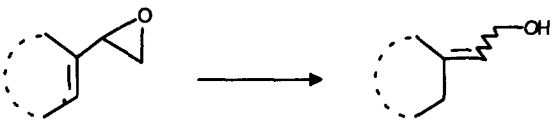


1

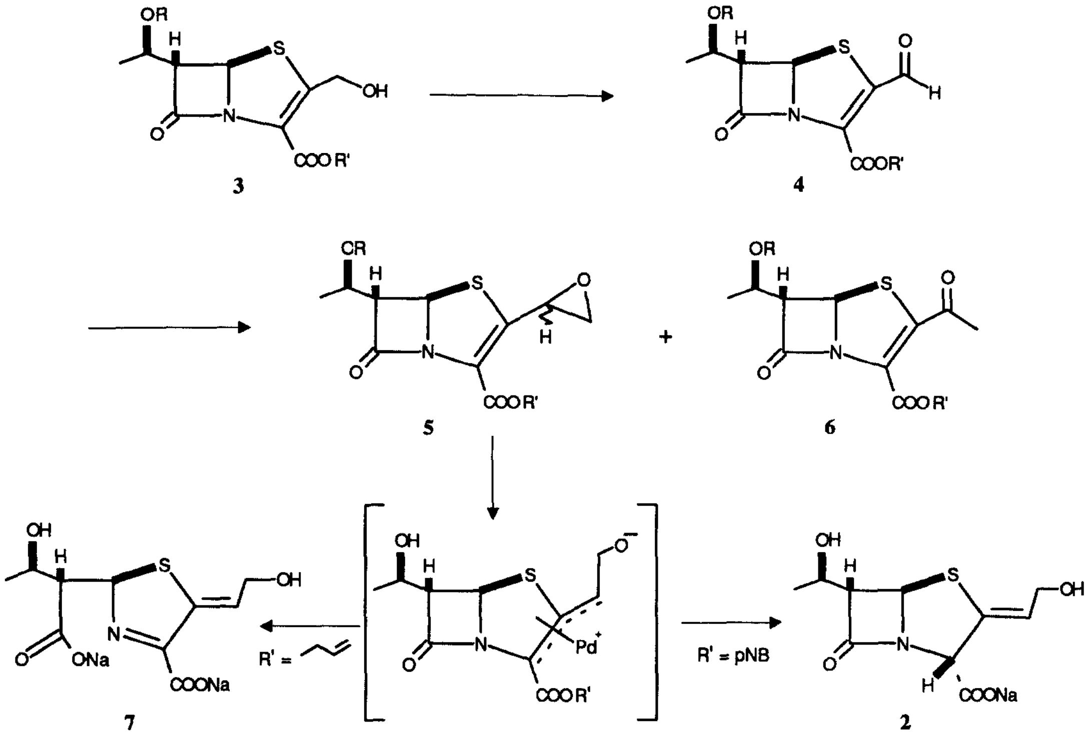


2

In the search for a straightforward approach toward this molecule we recognized its close relationship with a penem nucleus. In particular, we explored the synthesis of **2** *via* a palladium catalysed reductive rearrangement of the penem vinyl epoxide,[6] on the assumption that the bicyclic ring constrain would shift the double bond outside the ring (Scheme 1). Accordingly, our synthetic approach was based on the manipulation of the well-known 6-hydroxyethyl-2-hydroxymethyl penem intermediate 3[7] (Scheme 2).



Scheme 1



a, R = TBDMS, R' = $CH_2CH{=}CH_2$; **b**, R = TBDMS, R' = pNB; **c**, R = H, R' = pNB; **d**, R = H, R' = $CH_2CH{=}CH_2$

Scheme 2

MnO_2 oxidation of **3a**, **b** (CH_2Cl_2, room temperature, 3h) yielded the 2-formyl penems **4a**, **b** in almost quantitative yield.[8] Diazomethane treatment of **4a**, **b** (Et_2O, 0-5°C, 2h) gave a complex reaction mixture, consisting of the epimeric epoxides **5a**, **b** (30-40%) in admixture with the 2-acetyl derivative **6a**, **b** (70/30 **5**/**6** ratio).[9] When the epimeric oxiranes **5a**, **b** were subjected to TBAF/AcOH desilylation, extensive decomposition was observed and the desired deprotected derivatives **5c**, **d** were obtained only in low yields. Attempted deprotection of **5d** in the presence of catalytic amounts of $Pd(Ph_3P)_4$ (room temperature, 1h, THF, 1 eq Na 2-ethylhexanoate), yielded the Δ^3-thiazoline **7**[10,11] as the sole product. This result could be explained by the establishment of a π-allylpalladium complex, that, in the absence of a hydride source, underwent to β-lactam ring opening. The reducing agents reported so far for the Pd(0) catalysed rearrangement of vinyl epoxides (HCOOH, $LiBHEt_3$, $NaBH_4$, n-Bu_3SnH, polymethylhydrosiloxanes, alkylzinc derivatives bearing β-hydrogens)[6] were considered critical for our sensitive substrate. In the search of an alternative reagent, we addressed our attention to the use of H_2 and Pd/C as catalyst. The pNB derivative **5c** was selected for this new heterogeneous reductive rearrangement, since it was expected to undergo simultaneous ester removal. The low yield TBDMS deprotection was avoided by the use of the free 6-hydroxyethyl derivative **3c** as starting material. MnO_2 oxidation gave **4c** in high yield (90%). Rewardingly diazomethane treatment (Et_2O, 0-5°C, 2h) afforded the two epoxides **5c**[12] in a 1:1 mixture in 60% overall yields, with only minor amounts of ketone **6c** present (about 15%). The two epimers were separated at this stage and independently submitted to the final step. Remarkably, when the planned Pd catalysed reductive rearrangement was eventually carried out (catalytic amount of 10% Pd/C, H_2, room temperature, EtOAc, water, 1 mol eq. $NaHCO_3$, 3h), the configuration of the starting epoxide resulted to have no influence on the stereochemical outcome of the reaction, as both epimers gave the desired 6-hydroxyethylthiaclavulanic acid **2**[10] (40% unoptimized yield as sodium salt). In spite of the mild reaction conditions, still a certain amount of **7** was present in the reaction mixture (about 20%). The configuration at C3 of **2** was assigned by the chemical shift of the H3 proton, (5.19 ppm),[13] while the n.O.e. (14%) existing between the H3 and the vinylic proton proved the Z configuration at the double bond.

To our knowledge, this is the first example of a heterogeneous Pd/C, H_2 mediated vinyl epoxide rearrangement. While the exclusive formation of the Z isomer from both the epimeric epoxides seems to indicate the presence of a common π-allylpalladium complex, nevertheless further studies are needed to gain a deeper insight into the reaction mechanism. The work actually ongoing in our laboratories on the scope and limitations of this reductive rearrangement will also be reported in due time.

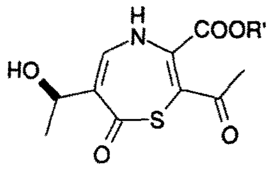
The *in vitro* β-lactamase inhibitory activity of **2** was assayed indirectly by determining the antibacterial activity[14] of ampicillin on β-lactamases producing strains, in the presence of two fixed concentrations of this compound. Even if **2** possessed a narrow inhibitory spectrum, a remarkable inhibition was observed on group V β-lactamases producing strains (Table 1) and, to a lesser extent, on group II.

Table 1. β-Lactamase Inhibitory Activity of 6-Hydroxyethylthiaclavulanic acid **2**.

Strains	β-Lactamase group[15]	MIC(μg/mL) Ampicillin	Ampicillin+ 20μg/mL **2**	Ampicillin+ 80μg/mL **2**
Staphylococcus aureus 39/2	II	1.56	<0.39	<0.39
Escherichia coli RP1	III	>100	>100	>100
Escherichia coli RGN	V	12.5	6.25	3.12
Escherichia coli R57b	V	100	6.25	1.56
Klebsiella aerogenes 1082E	IV	>100	>100	>100
Enterobacter cloacae P99	I	>100	>100	>100

References and notes

1. Cherry, P. C.; Newall, C. E. *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B.; Gorman, M. Eds.; Academic Press: London, 1982, 388.
2. Foglio, M.; Franceschi, G.; Errante, G. S.; Ballabio, M.; Arcamone, F. *Heterocycles* **1981**, *15*, 785.
3. Douglas, J. L.; Martel, A.; Caron, G.; Menard, M.; Silveira, L.; Clardy, J. *Can. J. Chem.* **1984**, *62*, 2282. Osborne, N. F. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1429.
4. Nishimura, S.; Nobuyoshi, Y.; Sasaki, H.; Matsumoto, Y.; Kamimura, T.; Sakane, K.; Takaya, T. *J. Antibiotics* **1990**, *43*, 114.
5. Pfaendler, H. R.; Neumann. T.; Bartsch, R. *Synthesis* **1992**, 1179.
6. Oshima, M.; Yamazaki, H.; Nisar, M.; Tsuji, J. *J. Am. Chem. Soc.* **1989**, *111*, 6280, and references cited herein.
7. Franceschi, G.; Perrone, E.; Alpegiani, M.; Bedeschi, A.; Zarini, F. *Penem Antibiotics*; Mitsuhashi, S.; Franceschi, G. Eds.; Springer-Verlag: Berlin, 1991, pp. 3-9.
8. Hanessian, S.; Bedeschi, A.; Battistini, C.; Mongelli, N. *J. Am. Chem. Soc.* **1985**, *107*, 1438.
9. Separation of the undesired 2-acetyl derivatives **6a**, **b** was performed at this stage. Anyway this purification resulted unnecessary, as these compounds upon treatment with TBAF underwent rearrangement to thiazepinone **8**, which could be easily separated from the reaction mixture.



8

10. ^{1}H NMR data for **2** and **7** are shown below: **2**, isolated as sodium salt by reverse phase column chromatography eluting with water: ^{1}H NMR(200 MHz, D_2O) δ ppm: 1.32 (3H, d, J = 6.4 Hz); 3.51 (1H, dd, J = 6.3 and 1.4 Hz); 4.12 (2H, dd, J = 6.3 and 1.0 Hz); 4.25-4.30 (1H, m); 5.19 (1H, d, J = 1.1 Hz); 5.35 (1H, d, J = 1.4 Hz); 5.88 (1H, dt, J = 6.3 and 1.1 Hz). **7** was isolated as disodium salt : ^{1}H NMR (200 MHz, D_2O) δ ppm: 1.26 (3H, d, J = 6.3 Hz); 2.69 (1H, dd, J = 7.4 and 9.0 Hz); 4.10-4.20 (1H, m); 4.24 (2H, dd, J = 6.1 and 1.7 Hz); 6.05 (1 H, m); 6.16 (1H, m).
11. Visentin, G.; Perrone, E.; Borghi, D.; Rizzo, V.; Alpegiani, M.; Bedeschi, A.; Corigli, R.; Rivola, G.; Franceschi, G. *Heterocycles* **1992**, *33*, 859.
12. The two epimers **5c** were separated and ^{1}H NMR spectra were taken for both separately. The absolute configurations were not assigned. Isomer 1, pale yellow amorphous solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 1.37 (3H, d, J = 6.3 Hz); 2.86 (1H, dd, J = 5.6 and 2.4 Hz), 3.13 (1H, dd, J = 5.6 and 4.3 Hz); 3.79 (1H, dd, J = 6.3 and 1.6 Hz); 4.20-4.30 (1H, m); 4.53 (1H, dd, J = 4.3 and 2.4 Hz); 5.25 (1H, d, J = 13.7 Hz); 5.45 (1H, d, J = 13.7 Hz); 5.57 (1H, d, J = 1.6 Hz); 7.60 (2H, m); 8.21 (2H, m). $[\alpha]_D$ = +12 (c 1.0, dioxane). Isomer 2: 1.37 (3H, d, J = 6.3 Hz); 2.97 (1H, dd, J = 5.3 and 2.6 Hz), 3.10 (1H, dd, J = 5.3 and 4.3 Hz); 3.77 (1H, dd, J = 6.3 and 1.7 Hz); 4.20-4.30 (1H, m); 4.60 (1H, dd, J = 4.3 and 2.6 Hz); 5.25 (1H, d, J = 13.6 Hz); 5.58 (1H, d, J = 13.6 Hz); 5.65 (1H, d, J = 1.7 Hz); 7.63 (2H, m); 8.23 (2H, m).
13. Osborne, N. F. *J. Chem. Soc., Perkin Trans. 1* **1980**, 150.
14. The antibacterial activity was determined by twofold dilution method on Mueller Hinton agar. Inocula were prepared from cultures in Mueller Hinton Broth, diluted to give approximately 10^4 cfu/spot. Plates were incubated for 18h at 37°C. MIC was the lowest drug concentration producing no visible bacterial growth. Compound **2** was added to ampicillin at fixed final concentrations of 20 and 80 μg/mL.
15. Richmond, M. H.; Sykes, R. B. *Adv. Microb. Physiol.* **1973**, *9*, 3188.