Tetrahedron Letters,Vol.29,No.50,pp 6611-6614,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

MICROBIAL TRANSFORMATIONS 11. REGIOSELECTIVE HYDROXYLATION OF ß-LACTAMS BY THE FUNGUS BEAUVERIA SULFURESCENS

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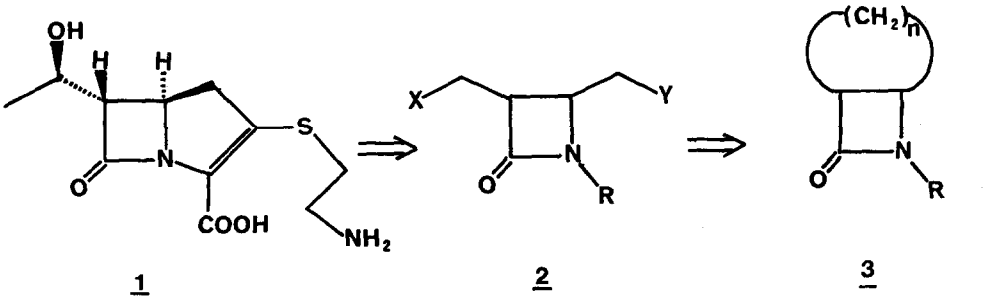
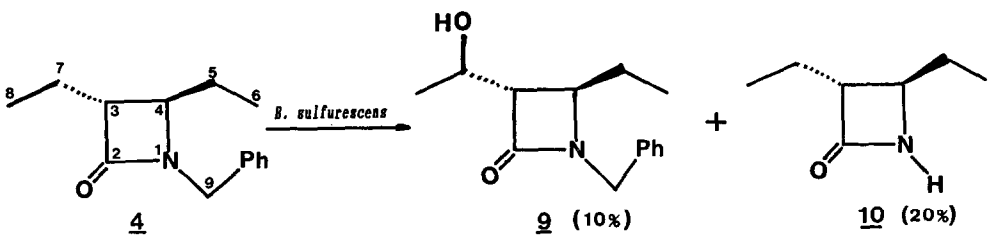
Abstract. The biohydroxylation of various mono or polycyclic ß-lactam derivatives by the fungus Beauveria sulfurescens (ATCC 7159) have been studied. The results obtained show that various monohydroxylated products can be obtained out of these transformations, most of these hydroxylations being highly regiospecific.

Owing to their outstanding pharmacological properties, ß-lactam derivatives are of utmost importance (1,2). Some interesting synthetic approaches to these products imply enzymatic (or microbiological) steps, leading to optically active compounds (3,4). As several compounds of this type, like for instance the important carbapenem derivative thienamycin 1, bear a free hydroxyl group on their carbon skeleton (1), we have been interested in studying ways allowing to achieve one-step hydroxylation of such products. Also, as developped previously for instance by OHNO and coll. (4), one strategy towards the synthesis of these compounds implies formation of the five membered ring starting from disubstituted ß-lactam building blocks like 2, which in turn can be obtained from bicyclic precursors of type 3. In the course of our work related to microbiological hydroxylations of non activated carbon atoms by the fungus *Beauveria sulfurescens* (5) we have been interested in studying the bioconversions of ß-lactam precursors which, to our best knowledge, have never been studied previously in this context.

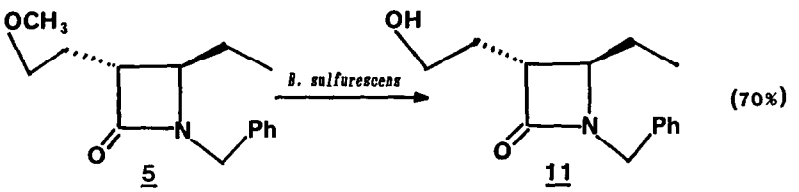
The racemic substrates, i.e. the monocyclic ß-lactams 4 and 5, as well as the bi and tricyclic substrates 6, 7 and 8 are readily available using the cycloaddition reaction of the corresponding olefins with N-chlorosulfonyl isocyanate (6), followed by benzylation of the lactam thus formed (7). The structures of the hydroxylated products (isolated yields are indicated on following scheme), obtained using the standard experimental conditions previously described (5), have been essentially determined using classical ^{1}H and ^{13}C NMR spectroscopy. As can be seen, it appears - first - that the biohydroxylation of all these substrates by the fungus does occur with fair to reasonnable yields - second - that, as noted previously on various compounds, the process appears to be highly regioselective, leading to essentially one single product in the case of the bridged tricyclic compounds 7 and 8. Also, in the case of 7, only the exo alcohol has been formed with complete stereoselectivity. It is also interesting to note the complete change of regioselectivity observed for substrate 4 compared to 5, 4 leading to hydroxylation on C_7 and C_9 whereas, in the case of 5, the only observed reaction very likely happens to be hydroxylation on the methoxy group, leading to an hemicetal intermediate, which is



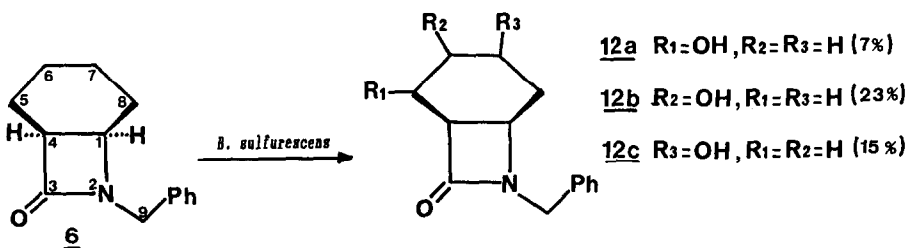
1 2 3



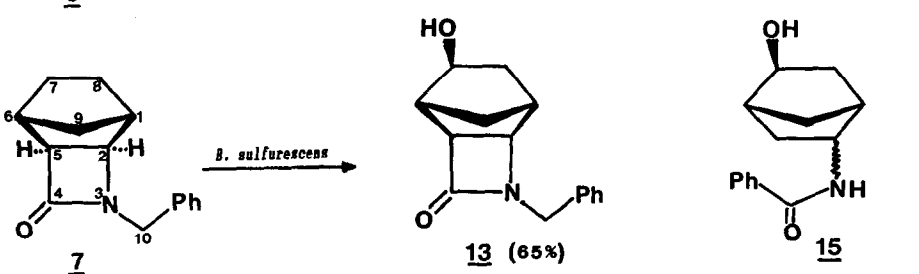
4 9 (10%) 10 (20%)



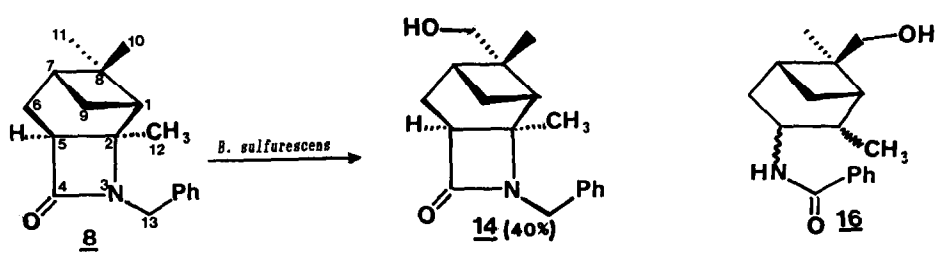
5 11



6



7 13 (65%) 15



8 14 (40%) 16

readily hydrolyzed to 11. Similarly, the major product obtained from 4 is 10, presumably formed by benzylic hydroxylation followed by hydrolysis of the aminocetal thus formed. Some benzaldehyde is indeed formed during this reaction. These results are presumably due to the higher reactivity of the hydrogen atoms linked to a carbon located on a hetero atom, and to the existence, in this fungus, of an enzymatic system able to achieved these hydroxylations as we have already observed previously on pyrrolidone type substrates (8).

The results obtained from tricyclic ß-lactams 7 and 8 compare interestingly with those we have observed previously in the course of our studies on norbornyl and pinane amide derivatives 15 et 16. Indeed, it appears that, in the case of 7, the fact that the nitrogen atom is linked to the same carbon atom as in the norbornane derivatives (9) leads to an identical regioselectivity for the hydroxylation process. On the other hand, the different localization of the nitrogen atom in 8, compared to that of the amide moiety in the pinane derivatives (10), orients the hydroxylation towards a different carbon atom. This observation is consistent with the fact that this nitrogen atom (i.e. the amide function) plays the role of an anchoring moiety of the substrate onto the enzymatic active site. Thus, a different localization of this atom results in a different regioselectivity for the hydroxylation. Finally, it is interesting to note that, formed from racemic substrates, the hydroxylated products display optical activity. However, because of the generally low optical rotations observed, we did not pursue determination of their enantiomeric excess.

As a conclusion, we have shown that biohydroxylation of various ß-lactam derivatives can be achieved by the fungus *Beauveria sulfurescens*. This allows preparation of several ß-lactams bearing a free hydroxy group, thus providing a chemical "handle" allowing chemical modifications on their carbon skeleton. These can therefore be valuable precursors of carbapenem or bactam type molecules, the introduced hydroxyl group allowing also covalent attachement of these models on various polymeric supports.

BIBLIOGRAPHIE

1/ R.W. RATCLIFFE and G. ALBERS-SCHONBERG in "*Chemistry and Biology of ß-lactam Antibiotics*". Vol.2, p. 227, Edts R.B. MORIN and M. GORMAN, Academic Press (1982)

2/ R. LABIA et C. MORIN ; *J. Antibiot.*, 37, 1104 (1984) ; J.E. BALDWIN ; *Tetrahedron*, 39, 2445 (1983).

3/ G.I. GEORG and H.S. GILL ; *J. Chem. Soc., Chem. Commun.*, 1433 (1985).

4/ H. KAGA, S. KOBAYASHI and M. OHNO ; *Tetrahedron Lett.* 29, 1057(1988).

5/ A. ARCHELAS, J.D. FOURNERON and R. FURSTOSS ; *J. Org. Chem.* 53, 1799 (1988) and related papers.

6/ J.K. RASMUSSEN et A. HASSNER ; *Chem. Rev.*, 76, 389 (1976).

7/ D. REUSCHLING, H. PIETSCH and A. LINKIES ; *Tetrahedron Lett.*, 615 (1978).

8/ A. ARCHELAS, R. FURSTOSS, D. SRAIRI and G. MAURY ; *Bull. Soc. Chim. Fr.*, 234 (1986).

9/ J.D. FOURNERON, A. ARCHELAS, B. VIGNE and R. FURSTOSS ; *Tetrahedron*, 43, 2273 (1987).

10/ A. ARCHELAS, J.D. FOURNERON, B. VIGNE and R. FURSTOSS ; *Tetrahedron*, 42, 3863 (1986).

SPECTROSCOPIC DATA

4 : IR (CHCl3) = 1740 cm^{-1} ν(C=O) ; ^1H 80 MHz NMR (CDCl3) : δ ppm = 0.80 (t, 3H, Me); 0.95 (t, 3H, Me); 1.0-2.0 (m, 4H); 2.85 (m, 1H, H-3); 3.1 (m, 1H, H-4); 3.95 and 4.6 (d, 1H, H-9); 7.23 (m, 5H, Ar.).

9 : IR (CHCl3) = 1760 ν(C=O) and 3450 ν(OH) cm^{-1} ; ^1H 80 MHz NMR (CDCl3) : δ ppm = 0.95 (t, 3H, CH3); 1.25 (d, 3H, CH3); 1.0-2.4 (m, 3H, H-5 and OH); 2.80 (m, 1H, H-3); 3.25 (m, 1H, H-4); 3.45 (m, 1H, H-7); 4.0 and 4.6 (d, 1H, H-9); 7.3 (m, 5H, Ar.).

10 : IR (CHCl3) = 1740 ν(C=O) and 3250 ν(N-H) cm^{-1} ; ^1H 80 MHz NMR (CDCl3) : δ ppm = 0.95 (t, 3H, CH3); 1.05 (t, 3H, CH3); 1.4-2.0 (m, 4H); 2.60 (m, 1H, H-3); 3.15 (m, 1H, H-4); 7.2 (broad s, 1H, NH).

5 : IR (CHCl3) = 1730 cm^{-1} ν(C=O) ; ^1H 80 MHz NMR (CDCl3) : δ ppm = 0.86 (t, 3H, H-6); 1.2- 2.2 (m, 4H); 2.86 (m, 1H, H-3); 3.14 (m, 1H, H-4); 3.29 (s, 3H, OCH3); 3.45 (t, 2H, H-8); 4.05 and 4.65 (d, 1H, H-9); 7.30 (m, 5H, Ar.). 11 : IR (CHCl3) = 1730 (C=O) and 3420 (OH) cm^{-1} ; ^1H 80 MHz NMR (CDCl3) : δ ppm = 0.85 (t, 3H, H-6); 1.0-2.1 (m, 4H); 2.80 (m, 1H, H-3); 3.1 (m, 1H, H-4); 3.4 (broad s, 1H, OH); 3.65 (t, 2H, H-8); 4.05 and 4.65 (d, 1H, H-9); 7.30 (m, 5H, Ar.).

6 : IR (CHCl3) = 1720 cm^{-1} ν(C=O) ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 1.2-2.0 (m, 8H); 3.14 (m, 1H, H-4); 3.62 (m, 1H, H-1); 4.09 and 4.54 (d, 1H, H-9, J_{gem}= 15 Hz); 7.28 (m, 5H, Ar.); ^{13}C NMR (CDCl3) : δ ppm = 16.9*(C-6); 18.9*(C-7); 19.6*(C-5); 23.0 (C-8); 44.4 (C-9); 47.0 (C-4); 50.1 (C-1); 127.4, 128.2, 128.6, 136.3 (Ar.); 170.3 (C-3).

12a : IR (CHCl3) = 1720 ν(C=O) and 3430 ν(OH) cm^{-1} ; ^1H 200 MHz (CDCl3) : δ ppm = 1.2-1.8 (m, 5H); 1.95 (m, 1H); 3.22 (m, 2H, H-4 and OH); 3.78 (m, 1H, H-1); 4.13 and 4.53 (d, 1H, H-9, J_{gem}= 15 Hz); 4.27 (m, 1H, H-5); 7.29 (m, 5H, Ar.) ; ^{13}C NMR (CDCl3) δ ppm = 15.2 (C-7); 23.1 (C-8); 28.8 (C-6); 44.5 (C-9); 50.9 (C-1); 55.1 (C-4); 65.4 (C-5); 127.8, 128.3, 128.8, 136.1 (Ar.) ; $[\alpha]_D^{21}$ = +0.8° (c = 2.5, CHCl3).

12b : IR (CHCl3) = 1720 ν(C=O) and 3420 ν(OH) cm^{-1} ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 1.2-2.0 (m, 5H); 2.10 (ddd, 1H, H-5, J= 14 Hz, J= 4.8 Hz, J= 4.8 Hz); 2.58 (m, 1H, H-4); 3.63 (m, 1H, H-1); 4.06 (m, 1H, H-6); 4.10 and 4.50 (d, 1H, H-9, J_{gem}= 15 Hz); 7.28 (m, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 20.6 (C-8); 27.1 (C-5); 29.2 (C-7); 44.5 (C-9); 45.9 (C-4); 49.4 (C-1); 64.3 (C-6); 127.7, 128.3, 128.8, 136.2 (Ar.); 173.2 (C-3) ; $[\alpha]_D^{21}$ = -4.5° (c = 2.3, CHCl3).

12c : IR (CHCl3) : 1720 ν(C=O) and 3430 ν(OH) cm^{-1} ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 1.3-2.2 (m, 6H); 3.21 (m, 1H, H-4); 3.7-3.9 (m, 3H, H-1, H-7 and OH); 4.16 and 4.52 (d, 1H, H-9, J_{gem}= 15 Hz); 7.3 (m, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 18.2 (C-5); 30.2 (C-6); 32.7 (C-8); 44.7 (C-9); 46.3 (C-4); 49.9 (C-1); 64.0 (C-7); 127.8, 128.4, 128.9, 136.0 (Ar.); 170.3 (C-3) ; $[\alpha]_D^{21}$ = +5.7° (c = 2.5, CHCl3).

7 : IR (CHCl3) : 1720 cm^{-1} ν(C=O) ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 0.9-1.2 (m, 3H); 1.4-1.6 (m, 3H); 2.19 (broad s, 1H, H-1); 2.44 (broad s, 1H, H-6); 2.92 (broad d, 1H, H-5, J= 4.4Hz); 3.27 (broad d, 1H, H-2, J= 3.6 Hz); 4.11 and 4.51 (d, 1H, H-10, J_{gem}= 15 Hz); 7.30 (m, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 24.6*(C-7); 27.2*(C-8); 30.9 (C-9); 34.5 (C-6); 36.2 (C-1); 44.4 (C-10); 56.9**(C-5); 57.1**(C-2); 127.6, 128.0, 128.4, 136.1 (Ar.); 168.6 (C-4).

13 : IR (CHCl3) : 1720 ν(C=O) and 3420 ν(OH) cm^{-1} ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 1.3-1.5 (m, 3H); 1.63 (d, 1H, J= 11.2 Hz); 2.19 (broad s, 1H, H-1); 2.37 (s, 1H, H-6); 2.54 (s, 1H, OH); 2.82 (broad d, 1H, H-5, J= 3.3 Hz); 3.21 (broad d, 1H, H-2, J= 3.5 Hz); 3.65 (m, 1H, H-7); 4.1 and 4.49 (d, H-10, J_{gem} = 15 Hz); 7.26 (m, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 27.1 (C-9); 35.7 (C-1); 37.3 (C-8); 42,4 (C-6); 44.7 (C-10); 53.2 (C-5); 56.2 (C-2); 72.2 (C-7); 127.7, 128.4, 128.7, 135.7 (Ar.); 167.7 (C-4) ; $[\alpha]_D^{21}$ = -2° (c = 2.5, CHCl3).

8 : IR (CHCl3) : 1715 cm^{-1} ν(C=O) ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 0.85 (s, 3H, H-11); 1.15 (d, 1H, J= 10 Hz); 1.23 (s, 3H, CH3); 1.28 (s, 3H, CH3); 1.8-2.0 (m, 4H); 2.16 (d, 1H, J= 14 Hz); 2.9 (d, 1H, H-5, J= 10 Hz); 4.10 and 4.3 (d, 1H, H-13, J_{gem}= 15 Hz); 7.28 (s, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 22.9*(C-12); 23.1*(C-11); 24.7**(C-6); 25,7**(C-9); 27.5 (C-10); 39.3 (C-8); 41.1 (C-7); 43.8 (C-13); 48.6 (C-1); 50,7 (C-5); 63.2 (C-2); 127.4, 128.4, 128.8, 136.6 (Ar.); 171.5 (C-4).

14 : IR (CHCl3) : 1715 cm^{-1} ν(C=O) and 3420 ν(OH) cm^{-1} ; ^1H 200 MHz NMR (CDCl3) : δ ppm = 1.15 (d, 1H, J= 10 Hz); 1.28 (s, 3H, CH3); 1.30 (s, 3H, CH3); 1.8-2.0 (m, 4H); 2.14 (broad d, 1H, J= 14 Hz); 2.9 (broad s, 1H, OH); 2.9 (d, 1H, H-5, J= 10 Hz); 3.40 (s, 2H, H-11); 4.10 and 4.27 (d, 1H, H-13, J_{gem}= 15 Hz); 7.27 (s, 5H, Ar.) ; ^{13}C NMR (CDCl3) : δ ppm = 21.7*(C-10); 22.6*(C-12); 24.1 (C-9); 25.3 (C-6); 39.3 (C-7); 42.6 (C-8); 43.8 (C-13); 47.6 (C-1); 62.9 (C-2); 65.7 (C-11); 127.4, 128.4, 128.7, 136.1 (Ar.); 171.3 (C-4) ; $[\alpha]_D^{24}$ = -21° (c = 2, CHCl3).

(Received in France 12 July 1988)