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MICROBIOLOGICAL TRANSFORMATIONS 2. HYDROXYLATIONS OF NON ACTIVATED CARBONS IN GLOBULAR TYPE AMIDES.

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Abstract : This paper describes the regioselective biohydroxylation of 7-aza-brendane and 6-azatwistane derivatives.

Among the numerous useful applications of enzymes to organic chemistry, biohydroxylation certainly counts as one of the most valuable, in that it allows regio, stereo and even enantioselective functionalisation of non-activated carbons (1). In particular, pioneering work on biohydroxylations previously reported was mainly about linear or monocyclic amides (2).

Our interest in bridged bicyclic or polycyclic nitrogen compounds (3), has led us to compare the results concerning the biohydroxylation of bridged bicyclic amides by *Beauveria sulfurescens* (ATCC 7159) with those obtained from the corresponding lactams (4). In order to extend this work, it was of interest to submit some globular (5) type amides and lactams to these hydroxylations, and particularly to determine :

- if molecules of this type, which may be of interest from the pharmacological point of view (6), could be considered as good substrates for the hydroxylating enzyme of this particular fungus ;

- if, due to the higher lipophilicity attributed to globular compounds one would observe a marked difference in the regio- and stereoselectivity of the hydroxylation or in the yields of the obtained alcohols ;

- and, finally, if we would, as previously reported (4), observe the same regioselectivity for compounds presenting an amide linkage and for the corresponding lactams.

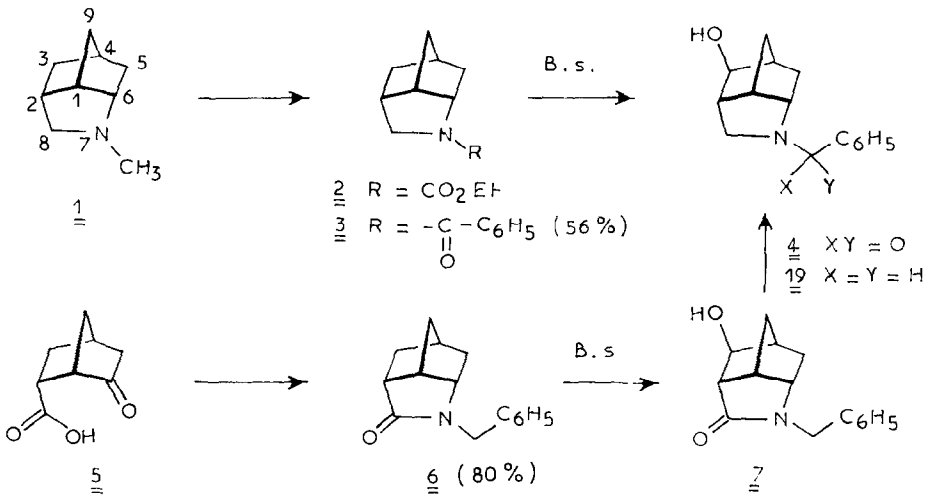
We wish to report here our results concerning the biohydroxylation of globular type amides 3 and 9 as well as of the corresponding lactams 6 and 15.

Compound 3 was prepared from N-methyl, 7-azabrendane (7). Reaction of 1 with ethylchloroformate in dry benzene affords urethane 2, which is successively treated with methyllithium and benzoyl chloride in dry THF to yield 3. The corresponding lactam 6 is obtained from ketoacid 5 (8) by a "one pot" reaction involving esterification, imine formation with benzylamine, hydrogenation over platinium oxide and spontaneous cyclisation of the

intermediate aminoester. Using identical reaction schemes, benzoyl azatwistane 9 was obtained from N-methyl, 6-azatwistane 8 (9), and lactam 15 was prepared from ketoester 14 (10) (cyclisation of the intermediate aminoester is achieved by pyrolysis).

Biohydroxylation of compound 3 leads, after normal work-up (filtration of the fungus and continuous extraction of the aqueous layer with chloroform) followed by analytic and preparative HPLC chromatography (silicagel), to alcohol 4 as the major product : (yield 53%) (11).

Similarly, biotransformation of 6 leads to tricyclic alcohol 7 as a single product (yield 50%).



Surprisingly enough, biohydroxylation of amide 9 gives off a mixture of three amidoalcohols and one amidoketone which are separated by HPLC chromatography and identified as being compounds 10, 11, 12 and 13 (respective proportions 58,28,10 and 4%).

On the other hand, bioconversion of lactam 15 leads to only two alcohols 16 and 17 and one ketone 18 (respective proportions 57,31 and 12%).

The structure and stereochemistry of the described compounds have unambiguously been determined by detailed study of their ^{1}H and ^{13}C NMR spectra, and have been confirmed by chemical transformations. Indeed LAH reduction of 4 and 7 affords 19, whereas oxidation of epimers 10 and 11 leads to 13 and that of 16 and 17 to 18.

The above described results clearly show :

- that, despite their sterically crowded skeleton, these globular type molecules are good substrates for this hydroxylating fungus ;
- that the yields and regioselectivities of these hydroxylations are not altered by the more lipophilic character of these compounds ;
- and, finally, that the geometrical features observed perfectly fit with our previous results, i.e. the carbonyl oxygen location does not influence the regioselectivity of the hydroxylation (4).

It is however noteworthy that the regioselectivity appears to be higher (formation of less by-products) for lactam models (where the amide moiety is blocked in the molecular framework) than for "external" amides, where two rotamers (rotation around the N-CO bond) preexist. It is also quite surprising that, whereas all previous results have shown that these hydroxylations were stereospecific (2,4) (as is also the case for azabrendane models 3 and 6), those of azatwistane compounds are apparently not. However, as some of these alcohols are obtained in optically active form, we do hope that the determination of their absolute configuration will allow us to shed some additionnal light on this apparent lack of stereospecificity.

REFERENCES AND NOTES.

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2 - See for instance R.A.JOHNSON, "Oxidation in Organic Chemistry", Part C, Acad.Press, p.131 (1978)

3 - R.FURSTOSS, R.TADAYONI and B.WAEGELL, J.Org.Chem., 42, 2844 (1977) ; Ph.MACKIEWICZ, R.FURSTOSS, B.WAEGELL, R.COTE and J.LESSARD, J.Org.Chem., 43, 3746 and 3750 (1978).

4 - R.FURSTOSS, A.ARCHELAS, B.WAEGELL, J.LE PETIT and L.DEVEZE, Tetrahedron Letters, 451 (1980).

5 - In order to distinguish between real "cage-type" molecules which may really encage some atoms or molecules (cryptates for instance), we do prefer the term "globular" which only describes a geometrical feature whithout implying any notion of content.

6 - See for instance a) K.AIGANO, Y.INAMOTO, N.TAKAISHI and Y.FUJIKURA, J.Med.Chem., 19, 536 (1976) b) C.RUNTI, M.DE NARDO, S.FABRISSIN, Il Farmaco Ed.Sc., 30, 260 (1975).

7 - R.FURSTOSS, R.TADAYONI and B.WAEGELL, Nouv.J.Chumie, 1, 167 (1977).

8 - a) R.W.ILES and W.S.WORALL, J.Org.Chem., 5233 (1961) b) M.D.DOWLE and D.L.DAVIES, Chem.Soc. Rev., 8, 171 (1979).

9 - R.FURSTOSS and B.WAEGELL, Tetrahedron Letters, 365 (1976).

10 - R.A.LEE, Tetrahedron Letters, 3333 (1973).

11 - All yields are calculated from the amount of compound obtained after HPLC purification.

12 - Owing to the presence of the two rotamers of the amide moiety (rotation around the N-CO bond), some hydrogen atoms do give two differentiated signals.

13 - These two doublets arise from the diastereomerically non equivalent benzylic protons.

* - *Beauveria sulfurescens* was previously classified as *Sporotrichum sulfurescens*, V.Beyma see : J.J.TAYLOR, Mycologia, 62, 797 (1970).

SPECTROSCOPIC DATA :

4 : IR ($CHCl_3$) = 3400 ν(OH) and 1615 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.40 (m, 5H) ; 4.38 and 3.85 (12) (dd, H_6, J_{H6-H1}= 5 Hz ; $J_{H6-H5\ exo}$= 8 Hz) ; 3.80 and 3.60 (dd, H_{8a}, J_{gem}= 11 Hz ; J_{H8a-H2}= 6 Hz); 3.64 and 3.45 (s, H_{3endo}) ; 3.58 and 3.30 (d, H_{8b}) ; 2.62 (m, H_1, J_{H2-H1}= 5 Hz) ; 2.40 (broad s, OH) ; 2.25 (m, H_2) ; 2.19 (m, H_4) ; 1.98 (d, H_{9a}, J_{gem}= 11 Hz) ; 1.95 and 1780 (m, $H_{5\ exo}$) ; 1.50-1.10 (m, 3H).

7 : IR ($CHCl_3$) = 3350 ν(OH) and 1675 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.30 (m, 5H) ; 4.75 (d, H_{10}, J_{gem}= 15 Hz) ; 3.95 (s, $H_{3\ endo}$) ; 3.92 (d, H_9) ; 3.70 (broad s, OH) ; 3.42 (m, H_6, $J_{H6-H5\ endo}$= 7.5 Hz ; J_{H6-H1}= 5 Hz ; $J_{H6-H5\ endo}$= 2 Hz) ; 2.91 (m, H_1, J_{H1-H2}= 4 Hz) ; 2.44 (m, H_2 and H_4) ; 2.20 (d, H_{9a}, J_{gem}= 11 Hz) ; 1.50 (m, $H_{5\ exo}$, $J_{H5exo-H4}$= 4 Hz) ; 1.36 (d, H_{9b}) ; 1.10 (d, $H_{5\ endo}$, $J_{H5\ endo-H5\ exo}$= 13.5 Hz).

10 (yield 53%) : IR ($CHCl_3$) = 3420 ν(OH) and 1615 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.4 (m, 5H) 4.5 and 3.64 (t , H_5, $J_{H5-H10\ exo}$= 6 Hz ; J_{H5-H4}= 6 Hz) ; 4.14 and 4.08 (m, H_9) ; 3.82 and 3.63 (dd H_{7b}, J_{gem}= 12 Hz ; J_{H7b-H2}= 4 Hz) ; 3.60 and 3.42 (d, H_{7a}) ; 2.46 (m, H_4) ; 2.38 (m, H_2) ;2.18 (m, H_1) ; 2.10-1.50 (m, 6H) ; 1.50 and 1.30 (m, $H_{10\ exo}$, J_{gem}= 12 Hz).

11 (yield 26%) : IR ($CHCl_3$) = 3400 ν(OH) and 1615 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.40 (m, 5H) ; 4.62 and 3.74 (t, H_5, $J_{H5-H10exo}$= 6 Hz ; J_{H5-H4}= 6 Hz) ; 4.24 (dd, H_9) ; 3.84 and 3.22 (dd, H_{7b}, J_{gem}= 12 Hz ; J_{H7b-H2}= 4 Hz) ; 3.65 and 3.45 (d, H_{7a}) ; 2.40 (m, H_4) ; 2.30-1.10 (m. 9H) .

12 (yield 9%) : IR ($CHCl_3$) = 3400 ν(OH) and 1610 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.20 (m, 5H) 4.66 and 4.02 (t, H_5, $J_{H5-H10\ exo}$= 6 Hz ; J_{H5-H4}= 6 Hz) ; 4.24 and 3.80 (dd, H_8) ; 3.85 and 3.25 (dd, H_{7b}, J_{gem}= 12 Hz ; J_{H7b-H2} = 4 Hz) ; 3.58 and 3.40 (d, H_{7a}) ; 2.38 (m, H_4) ; 2.30-1.10 (m, 9H).

13 (yield 4%) : IR ($CHCl_3$) = 1735 ν(C=O ketone) and 1620 ν(C=O lactam) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.44 (m, 5H) ; 4.80 and 3.98 (dd, H_5) ; 3.90 and 3.30 (dd, H_{7b}) ; 3.70 and 3.50 (d, H_{7a}) ; 3-1.10 (m, 9H).

16 (yield 52%) : IR ($CHCl)_3$ = 3380 ν(OH) and 1660 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.30 (m, 5H) 5.0 and 4.07 (d, $H_{benz.}$, J_{gem}= 15 Hz) ; 4.06 (dd, H_9, J_{H9-H8b}= 7.5 Hz ; J_{H9-H1}= 4.5 Hz) ; 3.40 (t , H_5, $J_{H5-H10\ exo}$= 5 Hz ; J_{H5-H4}= 5 Hz) ; 2.82 (t , H_2, J_{H2-H1}= 5.5 Hz ; $J_{H2-H3\ exo}$= 5.5 Hz) ; 2.30 (dd, $H_{3\ exo}$, J_{gem}= 12 Hz) ; 2.25 (m, H_1) ; 2.24 (s, OH) ; 2.0 (m, H_4) ; 1.90-1.60 (m, 2H) ; 1.50 (dd, $H_{10\ exo}$ and $H_{3\ endo}$, J_{gem}= 13 Hz) ; 1.40 (m, $H_{10\ endo}$).

17 (yield 28%) : IR ($CHCl_3$) = 3400 ν(OH) and 1660 ν(C=O) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.28 (m, 5H) 4.98 and 4.03 (d, $H_{benz.}$, J_{gem}= 15 Hz) (13) ; 4.22 (t , H_9, J_{H9-H8a}= 7.5 Hz ; H_{H9-H8b}= 7.5 Hz) ; 3.48 (t , H_5, J_{H5-H4}= 5 Hz ; $J_{H5-H10\ exo}$= 5 Hz) ; 2.80 (t , H_2, J_{H2-H1}= 5 Hz ; $J_{H2-H3\ exo}$= 5 Hz) ; 2.25 (dd,$H_{10\ exo}$, J_{gem}= 13 Hz) ; 2.0 (m, H_1) ; 2.0 (s, OH) ; 1.75 (dd, $H_{3\ exo}$, J_{gem}= 11 Hz) ; 1.7-1.2 (m, 4H).

18 (yield 11%) ; IR ($CHCl_3$) = 1735 ν(C=O ketone) and 1670 ν(C=O lactam) cm^{-1} ; 250 MHz NMR ($CDCl_3$) : δppm = 7.30 (m, 5H) ; 4.97 and 4.20 (d, $H_{benz.}$, J_{gem}= 15 Hz) (13) ; 3.72 (t , H_5, J_{H5-H4}= 5 Hz ; $J_{H5-H10\ exo}$= 5 Hz) ; 2.95 (t , H_2, J_{H1-H2}= 6 Hz, $J_{H1-H10\ endo}$= 6 Hz) ; 2.64 (m, H_1) ; 2.40 (m, 1H) 2.10 (dd, $H_{10\ exo}$, J_{gem}= 13 Hz) ; 1.74 (dd, $H_{10\ endo}$) ; 1.60 (dd, $H_{3\ exo}$).

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