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MICROBIOLOGICAL TRANSFORMATIONS. HYDROXYLATION OF NON ACTIVATED CARBONS IN BRIDGED BICYCLIC AMIDES.

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Abstract : The regio and stereoselectivity of the hydroxylations are described and the geometric requirements of the enzyme site are discussed.

Biohydroxylations are one aspect of the very interesting applications of enzymes to synthetic chemistry, as they allow regio, stereo and even enantioselective fonctionalisation of non activated carbons (1). Such reactions are of industrial value in the steroid field for instance (2).

Also, detailed studies on the interaction of low molecular weight substrates with enzymes represent one method for exploring the geometrical disposition and stereospecificity of the active site of the enzyme (3,4). However, the considerable number of degrees of freedom available to linear or monocyclic compounds may severely limit the utility of this approach. In order to circumvent this difficulty, we decided to study biohydroxylations of various bridged bicyclic and polycyclic amides. We here wish to report our results related to biohydroxylations of some derivatives of bridged bicyclic [3.2.1] and [2.2.2] azaoctanes.

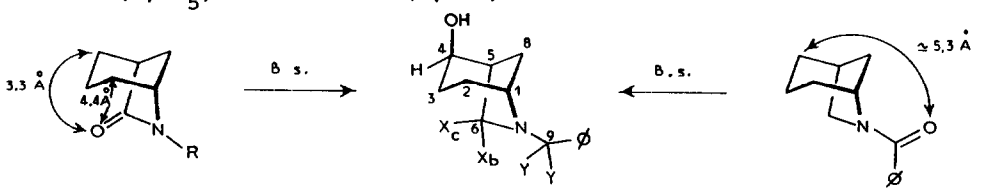
Compounds 1 to 6, prepared following classical ways (5,6) have been submitted to biohydroxylations by cultures of *Beauveria sulfurescens* (ATCC 7159). Preliminary results obtained by the method of WARBURG (7) showed that only the benzyl derivatives 3 and 6 led to a noticeable oxygen consumption. This selectivity for aromatic compounds may be due to a specific interaction of the phenyl group with an hydrophobic site of the operating enzyme - as for instance in the case of α-chymotrypsine (3) - and/or eventually to the lipophilic caracter of aromatics allowing penetration of the substrate through the cytoplasmic membrane of the fungi (8). Test experiments also show that biotransformation of these compounds reaches a maximum after about 90 hours of incubation. Leaving the reaction for a longer time did not increase the yield of products.

Biotransformation of lactam 3 leads, after normal work-up (filtration of the fungi and continuous extraction of the aqueous layer with chloroform), followed by analytic and preparative HPLC chromatography (silicagel), to a single product 7 (yield 45%); IR ($CHCl_3$) = 3620 ν(OH) and 1680 ν(C=O) cm^{-1};

250 MHz NMR ($CDCl_3$) : δppm = 7.3 (m, 5H) ; 4.9 (d, H_9, J_{gem}=15 Hz) ; 3.95 (d, H_9) ; 4.25 (s, H_{4e}) ; 3.6 (t, H_1, $J_{H1,H2e}$= 4 Hz ; $J_{H1,H8e}$= 4 Hz) ; 2.7 (t, H_5, $J_{H5,H4e}$= 5 Hz ; $J_{H5,H8e}$= 5 Hz) ; 2.45 (d, H_{8a}, $J_{H8a,H8e}$= 5 Hz) ; 1.95 (m, H_{8e}) and 1.4-1.8 (m, 5H).

Similarly, biotransformation of 6 leads to bicyclic alcohol 8 as a single product (yield 17,5%) ; IR ($CHCl_3$) = 3600 ν(OH) and 1655 ν(C=O) cm^{-1}; 250 MHz NMR ($CDCl_3$) : δppm = 7.25 (m, 5H) ; 4.55 (d, H_9, J_{gem}= 14 Hz) ; 4.45 (d, H_9) ; 4.28 (large d, H_5, $J_{H5,H6b}$= 10 Hz) ; 3.44 (m, H_1) ; 2.75 (m, H_4) ; 2.3 (m, 1H) ; 2.0 (m, H_{6b}) ; 1.9-1.4 (m, 4H) and 1.3 (m, H_{6a}).

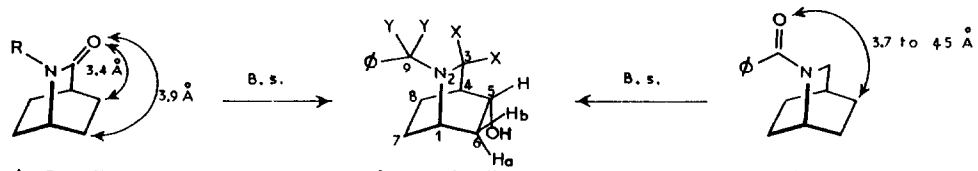
Finally, biohydroxylation of amide 9 (prepared from 1 by LAH reduction followed by condensation with benzoyl chloride) allows isolation of amido alcohol 10 ; (yield 50%) ; IR ($CHCl_3$) = 3620 ν(OH) and 1650 ν(C=O) cm^{-1}; 250 MHz NMR ($CDCl_3$)[13] : δppm = 7.4 (m, 5H) ; 4.52 and 3.9 (t, H_1,$J_{H1,H8e}$=5Hz; $J_{H1,H2e}$= 5 Hz) ; 3.95 and 3.85 (t, H_{4e}, $J_{H3e,H4e}$= 3 Hz, $J_{H4e,H5}$= 3 Hz) ; 3.65 and 3.45 (q, H_{6b},$J_{H5,H6b}$= 6 Hz, $J_{H6b,H6c}$= 11 Hz) ; 3.45 and 3.08 (d,H_{6c}); 2.52 and 2.44 (m, H_5) and 1.4-2.3 (m, 7H).



1 R = H
2 R = CH_3
3 R = $CH_2Ø$

7 XX = O ; Y = H
10 X = H ; YY = O
11 X = Y = H

9



4 R = H
5 R = CH_3
6 R = $CH_2Ø$

8 XX = O ; Y = H
13 X = H ; YY = O

12

The structures of these three alcohols as well as the stereochemistry of the hydroxyl function have unambiguously been determined by detailed studies of their 250 MHz NMR spectra including double irradiation experiments. However, in the case of amido alcohol 8, ^{13}C NMR studies were necessary to establish the exact stereochemistry of the OH group. These were performed on the basis of previous results described for bicyclo[2.2.2]octanol (9). Also, LAH reduction of compounds 7 and 10 lead to the same aminoalcohol 11. Noteworthy is the fact that, at our knowledge, no simple synthesis of 4-substituted, aza-6, bicyclo[3.2.1]octanes had previously been reported, and that the obtained products show optical activity.

Interestingly, broadly based studies concerning the introduction of hydroxy groups into derivatives of essentially linear or monocyclic amides with *Beauveria sulfurescens* have previously been reported (10). These results led to the proposal of a hypothetical enzyme-substrate model, and to the conclusion that a distance of 5,5 Å between the carbonyl oxygen and the hydroxylated carbon was the determining factor to the regioselectivity observed (10,11).

With these results in mind, it is interesting to compare the regio and stereoselectivity of the hydroxylation of compounds 3 versus 9, as well as of 6 versus 12 (hydroxylation of 12 has been described previously to give 13) (12).

These comparisons lead to two major remarks :

- First, the results obtained show that the location of the carbonyl group does not constitue a determining factor for the regioselectivity of the hydroxylation, i.e. one cannot orient the desired hydroxylation towards a particular site by moving the carbonyl function from one carbon atom to another, as is for instance the case in some steroïd hydroxylations (4).

- Second, examination of Dreiding models show that, in the models studied, the 5,5 Å distance between the carbonyl oxygen atom and the site of hydroxylation does not seem to be determining.

This is particularly noticeable for compounds 3 and 6 bearing the carbonyl function on the bicyclic framework. As can be seen from Dreiding models, hydroxylation takes place at a distance about 3,3 Å away from the carbonyl oxygen atom. Also, hydroxylation of 6 occurs at a distance of about 3,4 Å. Surprisingly enough, other carbon atoms situated at distances more compatible with the postulated 5,5 Å were available in each case[14].This may be due to the fact that the regioselectivity is determined by the nitrogen atom or the aromatic ring position, rather than by the carbonyl oxygen location as previously proposed (10,11).

Work is actually in progress in order to clarify this question, to determine the absolute configuration - i.e. the enantiospecificity - of the enzyme site, and to explore synthetic applications of these hydroxylations.

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13 - Owing to rotation around the N-CO bond, this molecule exists as a mixture of two rotamers. This leads to dedoubling of most of the signals and, by integration, allows to estimate the relative proportions of these rotamers to be about 70 and 30%.

14 - In the case of compound 6, only the aromatic carbon atoms are placed at about 5.5 Å away from the carbonyl oxygen atom.

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