Site Selective Oxidation of Some Azaspiroundecanes using *Beauvaria sulfurescens* ATCC 7159

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The micro-organism *Beauvaria sulfurescens* ATCC 7159 converted the bicyclic compounds (**5**), (**12**), (**17**), and (**19**) into the corresponding oxygenated products (**6**), (**13**), (**18**), and (**20**) respectively. The amide (**9**) gave the alcohols (**10**) and (**11**) under similar conditions while the isomeric substrate (**14**) gave equal amounts of the hydroxyamides (**15**) and (**16**).

The controlled functionalization of non-activated carbon centres in organic compounds is an ambitious goal that is attracting considerable attention. Much of the relevant work is aimed at introducing a hydroxy group to a position in a molecule remote from pre-existing heteroatoms. In recent years there has been some progress in producing novel, unnatural catalysts that effect regioselective hydroxylation of non-activated methylene units.[1] However, at the present time, biotransformations involving mono-oxygenase enzymes in whole cell systems are still the methods of choice which offer the organic chemist the best chance of success.

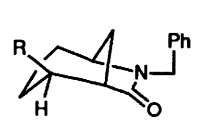
The hydroxylation of organic substrates using micro-organisms is well documented.[2] In particular the oxidation of steroids has received much attention.[3] The work of Furstoss *et al.* is noteworthy: on a number of occasions, the French team have used *Beauvaria sulfurescens* ATCC 7159 for the controlled hydroxylation of relatively simple amide substrates. For example,[4] the oxidation of a variety of azabicycloalkane derivatives *e.g.* (**1**) and (**2**) gave products (**3**) and (**4**) resulting from regioselective attack on the bicyclic molecules.

Unfortunately, when consideration is given to other substrates the site of oxidation is not easy to predict and so to try to shed more light on this important question we have subjected selected spiro-azabicycloundecanes to bio-oxidation using *B. sulfurescens* ATCC 7159.[5]

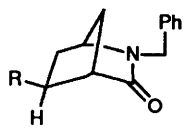
Results and Discussion

Incubation of the spiro-compound (**5**) with the micro-organism gave a single product resulting from oxidation of the carbocyclic ring. The yield of this compound (**6**) was an impressive 81% after crystallization. Oxidation of the alcohol (**6**) gave the ketone (**7**). The orientation of the hydroxy group relative to the piperidine ring was elucidated by NMR spectroscopy. Thus, reduction of the amide (**6**) to the amine (**8**) caused an upfield shift for the signal due to the protons H_a($\Delta\delta$ = 0.3) indicating that in the amide (**6**) the protons H_a fall within the shielding cone of the

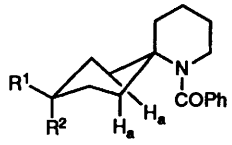
† Deceased 25 April 1990.



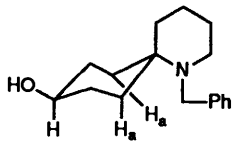
(1) R = H
(3) R = OH



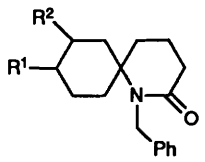
(2) R = H
(4) R = OH



(5) $R^1 = R^2 = H$
(6) $R^1 = OH, R^2 = H$
(7) $R^1R^2 = =O$

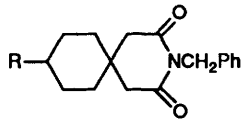


(8)



(14) $R^1 = R^2 = H$
(15) $R^1 = OH, R^2 = H$
(16) $R^1 = H, R^2 = OH$

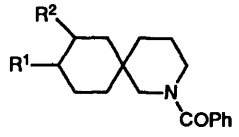
(17) R = H
(18) R = OH



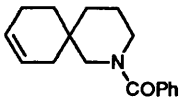
(19) R = H
(20) R = OH

carbonyl group. Irradiation of the signal due to the C*H*OH proton in the compound (**6**) gave rise to a nuclear Overhauser effect (5%) for the protons H_a, leading to the conclusion that the proton C*H*OH, the protons H_a, and the benzoyl moiety are present on the same face of the cyclohexane ring, and that the molecule adopts the conformation shown in the diagram.

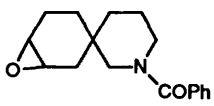
The azabicycloundecane (**9**) was partially oxidized using the *B. sulfurescens* sp. to give two products (**10**) and (**11**) in the ratio 2:1 (68% yield; 82% yield based on recovered starting material). Oxidation of both (**10**) and (**11**) gave the corresponding ketones in high yield. Not surprisingly,[6] the azaundecene (**12**) was converted into the epoxide (**13**) in good yield (74%). The ratio of diastereoisomeric epoxides was 10:1. In comparison, oxidation of the alkene unit in compound (**12**) using 3-chloroperoxybenzoic acid gave the same epoxides in the ratio 2:1. The situation of the hydroxy group in compounds (**10**) and (**11**) and the stereochemistry of the major epoxide formed from the alkene (**12**) could not be elucidated by spectroscopy.



(9) $R^1 = R^2 = H$
(10) $R^1 = OH, R^2 = H$
(11) $R^1 = H, R^2 = OH$



(12)



(13)

Microbiological oxidation of the piperidone (**14**) gave two products in equal amounts (64% total yield; 73% yield based on recovered starting material). Spectroscopic data showed the structures were (**15**) and (**16**). For compound (**16**) the orientation of the hydroxy group to the nitrogen-containing ring could not be conclusively defined; $LiAlH_4$ reduction of the amide (**15**) gave the alcohol (**8**), thus establishing the transannular relationship of the oxygen and nitrogen substituents.

Finally, the amide (**17**) gave a single product (**18**) in 31% yield (40% based on recovered starting material) on incubation with *B. sulfurescens*, while the imide (**19**) gave a poor yield (21%) of the hydroxy compound (**20**) under the same reaction conditions.

Several conclusions can be drawn from this work. First the unactivated carbocyclic ring in spiro-azaundecanes can be oxidized, often in excellent yield, using *B. sulfurescens* ATCC 7159. The site of oxidation occurs at C-9 largely or exclusively. The exact position of oxidation, and the ratio of products formed, depends on the position of the nitrogen atom and the situation of the carbonyl group associated with the amide moiety. However no obvious correlation emerged that linked the position and the type of amide unit to the site of oxidation. In our opinion still more work is necessary before this oxidative biotransformation becomes useful to a wider community.

Experimental

Biohydroxylation of N*-benzoyl-*1*-azaspiro*[5.5]*undecane using* Beauvaria sulfurescens (ATCC 7159).—The medium was corn steep liquor (20 g) and glucose (10 g) in water (1 dm^3) (adjusted to pH 4.85 with sodium hydroxide). The sterilized medium (2 dm^3) was inoculated with a 24 h old vegetative culture of *B. sulfurescens* and incubated with reciprocal shaking at 28 °C. After 72 h growth, an ethanolic solution of *N*-benzoyl-1-azaspiro[5.5]undecane (**5**; 400 mg, 1.56 mmol) was added to the culture. After an additional 48 h growth the mycelium was separated by filtration and washed with water. The filtrate was continuously extracted for 72 h with dichloromethane. The organic phase was washed with saturated sodium hydrogen carbonate solution, dried over magnesium sulphate, filtered and the solvent removed at aspirator pressure. The residual solid (**6**) was crystallized from ethyl acetate (343 mg, 81%); m.p. 184–185 °C (Found: C, 74.6; H, 8.4; N, 5.1%; M^+, 273. $C_{17}H_{23}NO_2$ requires C, 74.7; H, 8.5; N, 5.1%; M^+, 273); δ_H(250 MHz; $CDCl_3$), 1.3–1.9 (12 H, m), 2.2 (1 H, s), 2.9 (2 H, t), 3.2 (2 H, t), 3.9 (1 H, m); δ_C(62.9 MHz; $CDCl_3$), 17.29, 24.23, 28.23, 30.88, 31.18, and 44.86 (6 × CH_2), 59.17 (spiro C), 68.89 (CH), 127.00, 128.37, and 129.56 (3 × CH), 139.20 (C, Ph), and 173.41 (C, carbonyl); ν_{max}($CHCl_3$ solution), 3 880, 3 609, 2 932, 2 871, and 1 623 (CO), 1 371, 1 111, 1 048, 985, and 951.

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