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Synthesis Of Monohydroxylated 2-Azabicyclo[2.2.1]heptan-3-ones.

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Abstract: Oxymercuration/demercuration of 2-benzyl-2-azabicyclo[2.2.1]hept-5-en-3-one (**1**) and biohydroxylation of the corresponding saturated lactam (-)-(**8**) are compared as methods for the preparation of monohydroxylated 2-azabicyclo[2.2.1]heptan-3-ones. © 1997 Elsevier Science Ltd.

Carbocyclic nucleosides have been the focus of considerable synthetic endeavour over the last few years in view of their potential therapeutic uses, as both anti-tumour and anti-viral agents [(1)] , and more recently as potential DNA probes as a result of their expected greater metabolic stability. [(2)] Previously we have shown that it is possible to prepare 3'- deoxy carbocyclic nucleosides *via* the bromine mediated rearrangement of *N*-protected 2-azabicyclo[2.2.1]hept-5-en-3-ones in the presence of a suitable nucleophile, followed by hydrodebromination. [(3, 4)] However, this rearrangement has sofar not provided an access to the much sought after 2'-deoxy nucleosides, emphasizing that despite the ready availability of the bicyclic precursor 2-azabicyclo[2.2.1]hept-5-en-3-one, available in optically pure form *via* an enzyme mediated hydrolysis [(5)], introduction of a 5'-hydroxyl function appropriate for 2'-deoxycarbocyclic nucleosides is difficult, and requires a multi-step synthesis. Prompted by a recent report from Glaxo-Wellcome concerning the preparation of an alternative advanced cyclopentane intermediate for the preparation of 2'-deoxycarbocyclic nucleosides [(6)] we wish to report our preliminary results in this area. Particularly attractive to us was the reported direct biohydroxylation [(7)] of the saturated *N*-benzyl lactam (**8**); however the structure was only tentatively assigned as the required 5-*exo* hydroxy lactam from the little ^{1}H NMR data available. We report here a study of the products of oxymercuration/demercuration of the unsaturated *N*-benzyl lactam (**1**) enabling an umambiguous structural assignment of the hydroxylated products to be made together with our initial results concerning the biohydroxylation reaction.

Since direct oxymercuration [(8)] of 2-azabicyclo[2.2.1]hept-5-en-3-one failed to yield any useful product, oxymercuration of the *N*-benzyl lactam (**1**) was carried out. Thus treatment of the lactam (**1**), prepared using a modification of the procedure of reference 4, with mercuric acetate in THF/water followed

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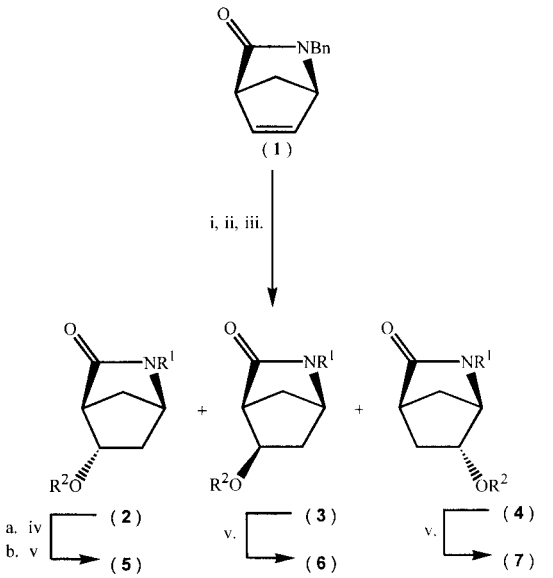
by reductive work-up and subsequent silylation [9] gave the silyl ethers (**2-4**, 45% overall yield) in a 2:1:2 ratio. Detailed ^{1}H NMR studies enabled the structures to be determined (selected data is included in the references). [10] Further studies relating to the origin of the 6-*exo*-alcohol will be reported in due course. The protected alcohol (**5a**, R^1 = H, R^2 = TBDMS) was prepared by debenzylation with sodium in liquid ammonia to give the potential 2'-deoxynucleoside precursor, while the *N*-benzyl alcohols(**5-7b**, R^1=Bn, R^2=H) were obtained by treatment with fluoride.

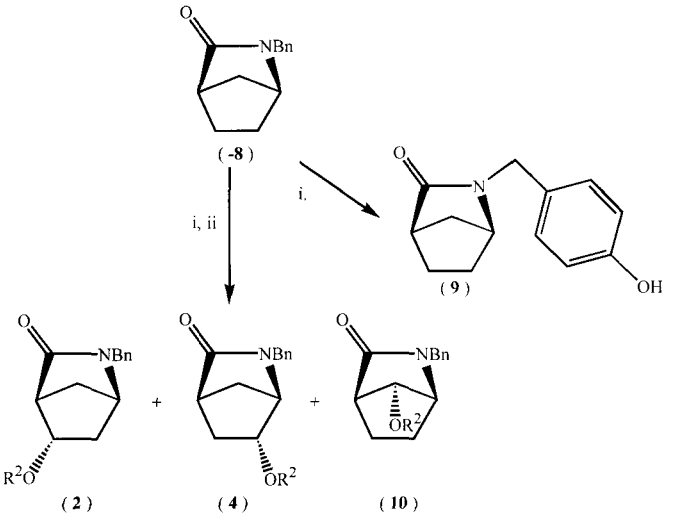


Scheme 1. i. $Hg(OAc)_2$, THF, H_2O; ii. $NaBH_4$; iii. ^{t}BDMSCl, imidazole; iv.Na/NH_3, v. TBAF/THF

The saturated lactam (-)-(**8**), available from lactam (-)-(**1**) by hydrogenation over palladium on carbon , was subjected to biohydroxylation by washed cell preparations of *Beauveria bassiana* ATCC 7159 (*B. sulfurescens*), previously shown to be a useful biocatalytic agent for the hydroxylation of unactivated carbons. [9] Subsequent analysis of the supernatant after centrifugation (capillary gc) showed the progressive formation of three hydroxylated products. One was the result of oxidation of the *N*-benzyl group to afford the 4'-hydroxybenzyl derivative (**9**), and the other two resulted from oxidation at C-5 (**2**) and C-6 (**4**) of the bicyclic lactam (ratio 5 : 1 respectively).(scheme 2) The structures of the latter two compounds were readily assigned by reference to the NMR spectra obtained for the oxymercuration products. The major product (5-*exo*) corresponds to a carbocyclic 2'-deoxynucleoside precursor.

In the biotransformation maximum yields (30 - 35%) of (**2**) were reached approximately 72 hours after addition of the substrate (1mg/ml). Additions of up to 3mg/ml of substrate were tolerated without any

significant toxic effects as judged by the time course and the extent of the biotransformation, although some variability, possibly due to changes in the nature of the culture, was noted between individual runs. On a larger scale the 7-hydroxylated lactam (**10**) was identified as a minor product (<5%) co-eluting with the 5-*exo* alcohol (**2**).



Scheme 2. i. *Beauvaria bassiana*; ii. ^{t}BDMSOTf, 2, 6-lutidine, 0°C

While the isolation of the three oxidation products indicates significantly less regioselective oxidation by the enzymes of *B. bassiana* ATCC 7159 than first reported by Archelas, [7, 11] we have demonstrated considerable enantioselection in that the microorganism hydroxylates (-)-(**8**) at four times the rate of (+)-(**8**). Other fungal strains have been shown to hydroxylate related bicyclo [2.2.1] substrates including *Absida orchidis* [12] and *Aspergillus awamori* [13], and so a screen of other fungal candidates including *Heminthosporium* sp. NRRL 4671 , *Mortierella isabellina* NRRL 1752, A*spergillus niger* ATCC 76837, *Penicillium lilacinium* ATCC 10114, *Cylindrocarpon radicicola* ATCC 11011, and *Beauveria densa* was carried out on the test substrate. Of those tested *M. isabellina* and *B. densa* were of most interest as both predominantly hydroxylated at the C-5 position.

Thus we have demonstrated through the use of chemically prepared standards that the biohydroxylation gives predominantly a compound that could be used as a precursor of 2-'deoxycarbocyclic nucleosides. This bioconversion has the potential to be developed into a synthetically useful procedure for the preparation of multigram quantities of the useful alcohol. Work is currently in progress to fully characterise the

biohydroxylation with *B. bassiana, M. isabellina*, and *B. densa* and to further demonstrate the practical use of this protocol to prepare hydroxylated materials from unactivated alkyl groups.

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10. Representative 300MHz ^{1}H NMR data for the 5-*exo*, 5-*endo*, and 6-*exo* silyl ethers (**2-4**, R^1 = Bn, R^2 = ^{t}BDMS) is shown below:-
    5-*exo* (**2**) 4.22 (1H, ddd, $J_{5\text{-endo-6-endo}}$ 6.6Hz, $J_{5\text{-endo-7-anti}}$ 1.3Hz, $J_{5\text{-endo-6-exo}}$ 2.2Hz, H-5*endo*), 2.02 (1/2H, app dd $J_{6\text{-endo-6-exo}}$ 13Hz, $J_{6\text{-endo-5-endo}}$ 6.6Hz, H-6*endo*), 1.94 (2H, m, H-7*anti*, H-7*syn*), 1.46 (1H, ddd, $J_{6\text{-exo-6-endo}}$ 13Hz, $J_{6\text{-exo-5-endo}}$ 2.2Hz, $J_{6\text{exo-4}}$ 2.1Hz, H-6*exo*).
    5-*endo* (**3**) δ ($CDCl_3$) 4.52 (2H, m including $J_{5\text{-exo-6-exo}}$ 8Hz, $J_{5\text{-exo-4}}$ 4.2Hz and $J_{5\text{-exo-6-endo}}$ 2.9Hz, H-5*exo* and CH_2Ph), 1.94 (1H, ddd, $J_{6\text{-exo-6-endo}}$ 13Hz, $J_{6\text{-exo-5-exo}}$ 8Hz and $J_{6\text{-exo-1}}$ 2.4Hz, H-6*exo*);
    6-*exo* (**4**) (see also reference 4) 3.76 (1H, dd, $J_{6\text{-endo-5-endo}}$ 6.6Hz, $J_{6\text{-endo-5-exo}}$ 2.2Hz, H-6endo), 2.00 (1H, dd, $J_{5\text{-endo-5-exo}}$ 13Hz, $J_{5\text{-endo-6-endo}}$ 6.6Hz, H-5*exo*), 1.50 (1H, ddd, $J_{6\text{-exo-6-endo}}$ 13Hz, $J_{5\text{exo-H-4}}$ 4.0Hz, $J_{5\text{exo-6-endo}}$ 2.2Hz, H-6*exo*).
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