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Synthesis and Microbial Hydroxylation of Some Azabicycloalkanes

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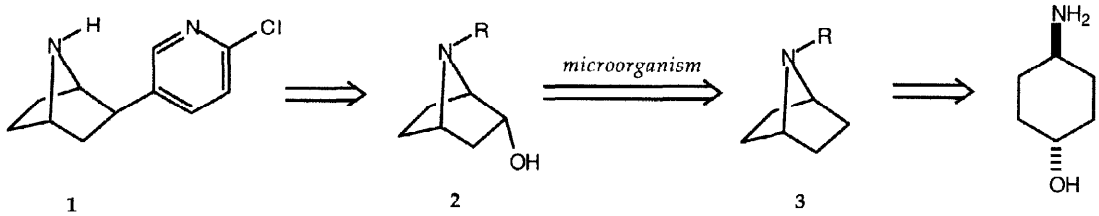
Abstract: *N*-Substituted 7-azabicyclo[2.2.1]heptanes have been synthesized in a short route. These compounds containing benzamide or benzenesulfonamide groups are good substrates for microbial oxidation of unactivated carbons by *B. bassiana*.

Since the structural elucidation of epibatidine (**1**) in 1992 by Daly and co-workers,[1] an enormous amount of effort has been devoted toward the synthesis of this alkaloid because of its potent analgesic activity, interesting mode of action and scarcity in nature.[2,3] Epibatidine's unusual structure consists of an *exo*-chloropyridinyl ring attached to a 7-azabicyclo[2.2.1]heptane skeleton. Although a large number of syntheses have appeared to date, a total synthesis involving a biocatalytic approach to this alkaloid has not yet been published (Scheme I).[4]

SCHEME I



1 2 3

The capability of microorganisms to oxygenate organic molecules has been exploited by Roberts,[5] Johnson[6] and others.[7] These groups independently reported that certain microorganisms were able to hydroxylate unactivated carbons of cyclic,[8] bicyclic,[9] tricyclic[10] and spiro molecules[11] when an appropriately substituted nitrogen was present in the substrate. Recently, Johnson described the microbial oxidation of several 7-azabicyclo[2.2.1]heptanes.[4] It was reported that oxidation of *N*-benzoyl-7-azabicyclo[2.2.1]heptane proceeded in poor yield; however, concurrent work in our laboratory showed this microbial oxidation to proceed in good yield.

Kibayashi,[12] Malpass,[13] and others[14] have prepared *N*-substituted 8-azabicylo[3.2.1]octanes (such as **4**, Figure 1). However, difficulty was encountered in the preparation of *N*-benzoyl-7-azabicyclo[2.2.1]heptane (**3a**) using the same methodology.[13] Hassner recently reported a four-step synthesis of *N*-alkyl-7-azabicyclo[2.2.1]heptane starting from commercially available mono-protected 1,4-cyclohexanedione.[15] In this communication, we present a shorter synthesis of some *N*-substituted-7-azabicyclo[2.2.1]heptanes (**3a-d**), the analogue compound **5**, and the microbial oxidation of bicyclics **3-5** using *Beauveria bassiana* (ATCC 7159).

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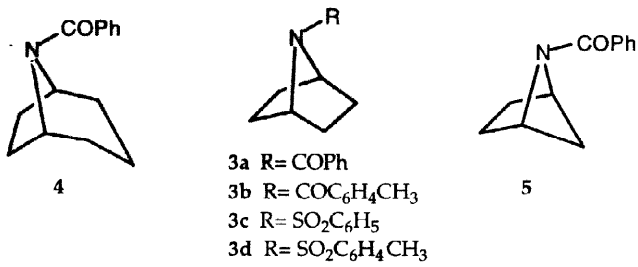


Figure 1. Substrates for microbial oxidation.

N-Benzoyl-8-azabicyclo[3.2.1]octane (**4**) was prepared following Kibayashi's procedure.[12] The analogue compound **5** was prepared following a modified protocol. Nitroso Diels-Alder adduct **7**, obtained from the *in situ* oxidation of benzohydroxamic acid with periodate and addition to cyclopentadiene,[16] was hydrogenated in the presence of palladium on carbon to give compound **8**, Figure 2. The nitrogen-oxygen bond was reductively cleaved with aluminum amalgam in THF-water to the corresponding *cis*-1,4-hydroxyamide **9** in excellent yield.[17] Treatment of alcohol **9** with thionyl chloride in the presence of triethylamine provided the *trans*-chloroamide **10** in good yield. Substitution by chloride occurred in good yields for the five and seven-membered ring series. However, elimination reaction was favored over substitution by chloride when *cis*-*N*-benzoyl-4-aminocyclohexanol was subjected to similar chlorination conditions.[12,13] The *trans*-chloroamide **10** was cyclized in the presence of potassium *tert*-butoxide to furnish the bicyclic molecule **5** in good yield.

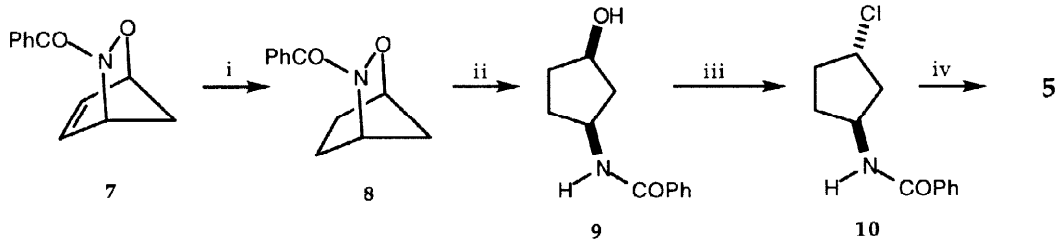
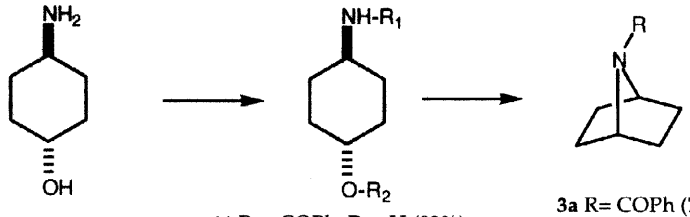


Figure 2. *Reagents:* i) H_2, Pd-C, EtOH, 95%; ii) Al(Hg), THF-H_2O (10:1), 95%; iii) $SOCl_2$, Et_3N, $CHCl_3$, 65%; iv) KO-*t*-Bu, DMF-C_6H_6 (1:1), 83%.

A short and efficient route to *N*-substituted 7-azabicyclo[2.2.1]heptanes **3a-d** was accomplished by a transannular nucleophilic displacement starting from commercially available *trans*-4-aminocyclohexanol hydrochloride (Figure 3). Treatment of a solution of *trans*-4-aminocyclohexanol in dichloromethane with 1.1 equivalents of benzoyl chloride in the presence of triethylamine provided hydroxybenzamide **11**. Treatment of hydroxybenzamide **11** with mesyl chloride in the presence of triethylamine in dichloromethane gave mesylate **12**. Addition of potassium *tert*-butoxide to mesylate **12** in benzene-DMF (1:1) gave the desired bicyclic compound **3a**. Preparation of *p*-toluenebenzamide **3b** was carried out using a similar protocol. This new approach to *N*-substituted 7-azabicyclo[2.2.1]heptanes required only three steps. Only two steps were required to form the bicyclic skeleton in compounds **3c-d**. The *trans*-4-aminocyclohexanol was treated with excess *p*-toluenesulfonyl chloride to form compound **15**, or benzenesulfonyl chloride to form compound **16**. Treatment of compounds **15** and **16** with potassium *tert*-butoxide in a 1:1 mixture of DMF-benzene provided the bicyclic compounds **3c** and **3d**, respectively.

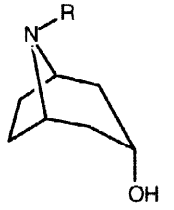


11 R_1= COPh, R_2= H (92%)
12 R_1= COPh, R_2= Ms (91%)
13 R_1= $COC_6H_4CH_3$, R_2= H (95%)
14 R_1= $COC_6H_4CH_3$, R_2= Ms (95%)
15 R_1= R_2= $SO_2C_6H_5$ (66%)
16 R_1=R_2= $SO_2C_6H_4CH_3$ (73%)

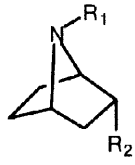
3a R= COPh (73%)
3b R= $COC_6H_4CH_3$ (65%)
3c R= $SO_2C_6H_5$ (94%)
3d R= $SO_2C_6H_4CH_3$ (89%)

Figure 3. Short Synthesis of *N*-substituted 7-Azabicyclo[2.2.1]heptanes.

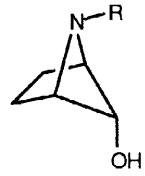
Stereospecific microbial hydroxylation of *N*-benzoyl azabicycloalkanes **3a**, **4**, and **5** using *B. bassiana* occurred in fair to good yields at unactivated carbons, furnishing *endo*-alcohols **17a**, **18** and **19** respectively, as shown in Figure 4. A mixture of rotamers was observed by NMR spectroscopy for both substrates and products due to the double bond character of the amide group. In order to facilitate their structural elucidation, metabolites **17a** and **18** were reduced with lithium aluminum hydride to their corresponding *N*-benzylamine derivatives, **20** and **21** respectively. ^{1}H- and ^{13}C-NMR spectra of *N*-benzylamine **20** were identical to those reported previously by Fletcher.[3] Desymmetrization of **3a** and **3b** to alcohols **17a** and **17b** both proceeded in 22% e.e., as determined by HPLC analysis of each Mosher ester derivative. Some desymmetrization of benzenesulfonamide **3c** to alcohol **17c** was also observed (18% e.e., determined by ^{1}H-NMR spectra of its Mosher ester).



18 R= COPh (49%)
21 R= CH_2Ph (98%)



17a R_1= COPh, R_2= OH (56%, 22% ee)
17b R_1= $COC_6H_4CH_3$, R_2= OH (8%, 22% ee)
17c R_1= $SO_2C_6H_5$, R_2= OH (57%, 18% ee)
17d R_1= $SO_2C_6H_4CH_2OH$, R_2= H (34%)
20 R_1= CH_2Ph, R_2= OH (98%)



19 R= COPh (30%)

Figure 4. Microbial hydroxylation using *B. bassiana* and reduction of benzamides with $LiAlH_4$.

Microbial oxidation of *p*-toluenesulfonamide **3d** occurred at the benzylic carbon to give **17d** as previously observed by Johnson.[4] Interestingly, oxidation of *p*-toluenebenzamide **3b** and benzenesulfonamide **3c** occurred exclusively on the remote unactivated carbon (**17b** and **17c**, respectively). Enantiomeric excess of hydroxysulfonamide **17c** was not found to be better than those of hydroxybenzamides **17a-b**. Other new substrates are being prepared to gain a better insight into these microbial oxidations.

In summary, we have presented a short synthesis of *N*-substituted azabicycloalkanes by a transannular nucleophilic displacement. The synthesis of *N*-benzoyl-7-azabicyclo[2.2.1]heptane was accomplished in only three steps from *trans*-4-aminocyclohexanol. Benzamides and benzenesulfonamides are both good *N*-substituents for facilitating these oxidations with *B. bassiana*. These oxidized microbial derivatives are useful intermediates for the synthesis of epibatidine and other analogues. A total synthesis of the nicotinic analgesic epibatidine will be reported in due course.

Biotransfomation Procedure. A culture of *Beauveria bassiana* (ATCC 7159) was obtained from the American Type Culture Collection and transferred onto sabouraud maltose agar slants using the techniques described by ATCC and provided with the culture. The cultures were grown at 28 °C for 7 days, sealed and stored at 4 °C. Subculturing was performed every two weeks, with cultures ready for use after 4 days of growing at 28 °C. Stage I cultures were grown from agar slants in 25 mL of medium A or medium B in 125 mL shake flasks at 250 rpm and 28 °C for 72 hours. Stage II cultures were grown from Stage I cultures in 200 mL of medium A or medium B in 1-L shake flasks. After 24 hours of growth, the substrate (100 mg per flask) was added as a solution in either 95% ethanol or DMF. After 5-7 days of shaking the cells were removed by vacuum filtration. After extracting the filtrate with CH_2Cl_2 (2-3 x 200 mL), the combined organic layers were dried with brine and Na_2SO_4, filtered and concentrated to a crude oil. The oil was flash chromatographed on silica gel to give the hydroxylated product. Medium A: 20 g of D-glucose, 5 g of yeast extract (Difco), 5 g of soybean meal (Victoria Feed Co.), 5 g of NaCl and 5 g of K_2HPO_4 per liter of water, pH adjusted to 5.0. Medium B: 20 g of corn steep liquor (Sigma), 10 g of D-glucose per liter of water, pH adjusted to 5.0. Media was sterilized by autoclaving 15 minutes (small flasks) or 20 minutes (large flasks) and allowed to cool to room temperature before inoculation.

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