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Biomimetic Synthesis of Nitraramine

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The synthesis of a possible biosynthetic precursor (9) of nitraramine (1) is described, utilizing N-Boc-piperidone as an equivalent of didehydroperipederine 4. Heating this reactive, achiral intermediate 9 in aqueous solution results in the stereoselective formation of natural nitraramine via three successive cyclization reactions. Since nitraramine and several other Nitraria alkaloids are obtained from Nitraria species in racemic form, this synthesis provides additional support for our hypothesis of non-enzyme-catalyzed formation of these alkaloids.

Alkaloids containing a 3-spiropiperidine structural unit are exclusively obtained from plants of the Nitraria genus. The Nitraria spiroalkaloids that contain 10 carbon atoms such as nitramine (2) and isonitramine (3) are most likely biogenetically derived from two piperidine synthons.1-2 Nitraramine (1) was isolated in 1975 from Nitraria schoberi, and the unusual structure of this considerably more complicated alkaloid was established by X-ray analysis in 1985.4 Nitraramine is a 15 carbon-atom-containing spiroalkaloid and is probably derived from three piperidine equivalents. Three boat-formed rings in the center of the molecule form an oxazabicyclooctane ring system, which is surrounded by three chair-formed six-membered rings, thus creating a completely rigid structure.

1 Nitraramine

2 Nitramine

3 Isonitramine

Remarkable is the racemic form in which nitraramine and several other Nitraria alkaloids occur in nature. In a recent publication Heathcock and co-workers synthesized petrosin, a racemic bisquinolizidone sponge alkaloid containing eight stereocenters. They nicely demonstrated that postbiosynthetic equilibriums are responsible for the racemic form in which petrosin is isolated from its natural source. Nitraramine (1), however, is a configurationally stable molecule6 and racemization via retroreactions is unlikely. Racemization experiments with enantiomerically pure material should give additional information in this respect; however, separation of nitraramine into enantiomers was unsuccessful until now.7 After some earlier synthetic approaches8 we describe here a synthesis that is based on the retrobiogenetic analysis we recently proposed and we will show that three successive cyclization reactions smoothly take place, once the reactive precursor anticipated is obtained. The ease with which this process occurs chemically (water-reflux) combined with the racemic form of natural nitraramine give an indication that no enzymes are involved in the stereochemically important spirocyclization reaction (Scheme 4). Another example of imine/enamine based cyclization reactions of achiral precursors in aqueous solution was recently provided by our biomimetic synthesis of the indole alkaloids nitrarine and nitramidine, also obtained as racemates form from Nitraria plants.

Biogenetic Hypothesis. Distinction should be made between the biosynthetic origin of these Nitraria alkaloids and for instance histrionicotoxine, which contains a 2-spiropiperidine ring system. A clear relationship exists between the Lupine alkaloids and those of the Nitraria family (Scheme 1). Labeling studies9 in Lupine species have established dipiperidine (tetrabromohydroxybomusine) as the biogenic precursor for piperidine alkaloids such as lupinine and sparteine. Although lysine, via cadaverine, is shown to be the precursor of 5, the existence of the instable didehydropiperidine 410 as an intermediate has not been confirmed. An alternative ring opening of the saturated piperidinering in 5 leads to 6, providing aldehyde 7 after oxidative deamination. An indication for this ring opening reaction is found in aqueous solutions of synthetic 511 (dihydrobromide), showing the presence of about 30% of the open form 6 according to 1H-NMR spectroscopy. Condensation of 7 with the enamine form of didehydropiperidine 4 gives precursor 8, which is probably in equilibrium with the

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7 We attempted resolution of nitraramine by crystallization with 1 and 2 equivs of the following reagents: (S)-(+)-mandelic acid; (1R)-(-)-10-camphorsulfonic acid; (1R)-(-)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate.
Biomimetic Synthesis of Nitraramine

Scheme 1. Biogenetic Hypothesis

Lysine → \[\text{didehydropiperidine 4}\]

dimerization

[\text{tetrahydroanabasine 5}] → [\text{Nitraramine}]

Corresponding \(\alpha,\beta\)-unsaturated imine 9. Conversion of this achiral precursor 9 into nitraramine will be discussed in the chemistry part.

Chemistry. Initially our biomimetic synthesis was performed by condensation reactions of glutaric aldehyde 10b with in situ-generated didehydropiperidine 4 in aqueous solution. Although these reactants have the correct oxidation state to obtain nitraramine, the capricious reactivity of glutaric aldehyde caused several undesired side reactions. Protection of one of the aldehyde functionalities in glutaric aldehyde gave acetal 10a, which was submitted to condensation reactions with didehydropiperidine 4 according to a literature procedure, describing the synthesis of 3-alkylidene substituted piperidines from aromatic and aliphatic aldehydes.\(^{(12)}\) None of the anticipated ene-imine product 11a could be obtained, however, due to limited stability of the imine functionality. Next we switched to N-Boc-piperidinone 12,\(^{(13)}\) a stable piperidine equivalent that can be converted efficiently into 4 via a reduction/deprotection sequence.\(^{(14)}\) The lithium enolate of N-Boc-piperidinone 12 was alkylated with aldehyde 10b and without isolation, the resulting mixture of diastereomeric alcohols was converted into \(\alpha,\beta\)-unsaturated lactam 13 (\(E:Z = 15:1\)) via mesylation and triethylamine-catalyzed elimination. This elimination effectively "protects" the OH during the next alkylation step. The acetal functionality of 13 was hydrolyzed with PPTS as catalyst, and the aldehyde 14 was alkylated with a second equivalent of the lithium enolate of 12 to give 15, representing the carbon framework that is required for the cyclization reactions. Water elimination as described for 13 produced the symmetrical dimer 19 (Scheme 3), the lactam analogue of 9. Adjusting the oxidation state of both piperidine carboxyls in 15 turned out to be problematic. Our initial goal, protection of both hydroxyls and the double bond in the form of pyran 20 (Scheme 3) was not possible since base-catalyzed conjugate addition of the alcohol function in


Among the more selective reducing agents lithium tri-
the Boc-substituents in workup the unsaturated hydroxypiperidine ring pre-
the unsaturated lactam moiety followed by 1,a-reduction 1a~tams.l~ In our situation both lactam carbonyls of

tions at pH formed at reflux temperature in buffered aqueous solu-

were reduced, leading to the unstable precursor 16

complete overreduction of the a,p-unsaturated lactam to is suitable for most N-Boc-piperidone reductions8 worked

form the corresponding ring opened allylic alcohol 40

Scheme 1.

15 could not be accomplished. NaBH₄ in methanol, which is suitable for most N-Boc-piperidone reductions8 worked well for the saturated lactam part of 15, but resulted in complete overreduction of the α,β-unsaturated lactam to form the corresponding ring opened allylic alcohol 17. Among the more selective reducing agents lithium triethylborohydride is superior for reductions of N-acyl lactams.15 In our situation both lactam carbonyls of 15 were reduced, leading to the unstable precursor 16 in moderate yield. It should be noted that after aqueous workup the unsaturated hydroxy piperidinering preferred the conjugated aldehyde form. 1,4-Reduction of the unsaturated lactam moiety followed by 1,2-reduction to form 18 lowered the yield. Acid-catalyzed removal of the Boc-substituents in 16 was performed with TFA in dichloromethane, leading to symmetrical dimer 9 most probably via elimination of water from 8 as is shown in Scheme 1.

Cyclization reactions with 9 (Scheme 4) were performed at reflux temperature in buffered aqueous solutions at pH 7, leading directly to nitramine as the only isomer. Spirocyclization of 8 completes the carbon-framework, and via cyclohexane ring-inversion of 21 the required 1,3-diaxial conformation for the two final acetal-forming steps is obtained. Although stereoisomerism is possible, especially at C1, none of these compounds was observed in ¹H NMR spectra of crude reaction mixtures. It should be noted that 1-epi-nitramine was recently isolated from Nitraria billardieri by Quirion et al. The moderate yield of the last step may be caused by a cleavage reaction, which actually represents a reversion of the proposed biosynthesis. A literature example of such a hydrolytic ene-imine cleavage reaction can be found in the biomimetic deethylation of geissoschizine derivative 22 to 23 as described by Lounasmaa et al. (Scheme 5).

Identification of nitramine was possible by comparison of ¹H and ¹³C NMR spectra with data from the literature.17,18 Extensive 2D NMR-spectroscopy was performed on both nitramine and its mono-hydrochloride, resulting in a partial revision of the literature chemical shift assignments.

In summary, it was demonstrated that water at neutral pH is an effective catalyst for biomimetic cyclization reactions with the imines and enamines that might be present in Nitraria species. Synthesis of the appropriate precursors, which in vivo would require several redox-enzymes, was accomplished with N-Boc-piperidone as a didehydropiperidine equivalent.

### Experimental Section

#### General Information

NMR spectra were obtained from a 400 MHz Bruker spectrometer. Thin layer chromatography (TLC) was performed on silica gel-coated plastic sheets. Chromatography refers to flash chromatography on silica gel (0.20–0.075 mm). When ammonia-containing eluents were used, the silica gel was pretreated with this eluent. Chromatography refers to flash chromatography on silica gel (20 cm, PE 60/80/EtOAc 3/1; TLC-spots were made visible with anisaldehyde/sulfuric acid). ¹H and ¹³C chemical shift assignments.

![Diagram](image-url)
workup (ether) yielded the crude alcohol, which was dissolved in a mixture of dry toluene (50 mL) and triethylamine (8.3 mL, 60 mmol). Methanesulfonyl chloride (3.5 mL, 45 mmol) was added dropwise at 0 °C, and the mixture was allowed to warm to room temperature. Additional triethylamine (13.8 mL, 100 mmol) was added and the mixture was refluxed during 5 h. Workup and chromatographic purification (PE 60/80/EtOAc 3/1) gave first impure 13 Z-isomer (0.6 g, 4%) and next 13 E-isomer (8.75 g, 62%) both as a syrup. Z-isomer: 1H NMR (CDCl3) δ 5.82 (t, J = 7.1 Hz, 1H), 6.56 (d, J = 15 Hz, 2H), 2.63 (m, 2H), 2.12 (m, 4H), 1.88 (m, 4H), 1.55 (m, 2H). E-isomer: 1H NMR (CDCl3) δ 7.13 (s, 1H), 6.58 (m, 2H), 4.18 (bs, 2H), 4.05 (t, J = 7.2 Hz, 1H), 3.87 (t, J = 5.4 Hz, 1H), 3.71 (t, J = 5.4 Hz, 1H), 3.48 (t, J = 7.1 Hz, 1H), 2.78 (m, 2H), 2.48 (m, 4H), 1.86 (m, 4H), 1.52 (m, 2H), 1.27 (m, 4H). 

Additional triethylamine (13.8 mL, 100 mmol) was added and the mixture was refluxed in a mixture of dry toluene (1 h) and quenched with saturated NH4Cl solution. Extractive isomer: 'H NMR (CDCl3) δ 5.82 (t, J = 7.1 Hz, 1H), 6.56 (d, J = 15 Hz, 2H), 2.63 (m, 2H), 2.12 (m, 4H), 1.88 (m, 4H), 1.55 (m, 2H). 

PPTS (0.12 g) in a mixture of water 1 mL) at 20 °C during 4 days. The reaction mixture was drying yielded 14 (0.56 g, 2 mmol) in THF was added at -78 °C, and the reaction mixture was stirred at this temperature during 2 h. Extractive workup (NH4Cl, ether) and chromatography (EtOAc:PE 60/80/211) gave nitraramine (17) 6.90 (m, 1H), 4.1 (b, 1H), 3.76 (m, 1H), 3.7-3.46 (m, 8H), 2.5-1.5 (m), 1.48 (s, 9H), 1.37 (s, 9H), 1.24 (s, 9H), 1.18 (s, 9H), 1.06 (m, 9H), 0.93 (m, 9H), 0.82 (m, 9H), 0.68 (m, 9H), 0.57 (m, 9H), 0.46 (m, 9H), 0.35 (m, 9H), 0.25 (m, 9H), 0.14 (m, 9H), 0.03 (m, 9H), 0.02 (m, 9H), 0.01 (m, 9H). IR (CHC13) 3500 (br), 1757, 1680-1720, 1620 cm-', HRMS obs mass 281.1649, calcd for C15H23O4N: 281.1570.

Lithium triethylborohydride (3.8 mL of a 1 M solution 20 °C) was added to a mixture of 14 (5.5 mmol) at 0 °C. After 15 min at 0 °C the mixture was stirred at room temperature for 30 min and evaporated in vacuo (bath T < 25 °C). The oily residue (9 TFA salt) was dissolved in CCl4 by the addition of CD30D (2 drops): 1H NMR (CDCl3) δ 8.43 (bs, 2H), 6.87 (bt, 2H), 3.65 (m, 4H), 2.42 (m, 4H), 1.98 (m, 4H), 1.74 (m, 2H); 13C NMR (CDCl3) δ 169.2, 129.8, 79.9, 73.32, 72.82; IR (CHC13) 3600-3200 (br), 1685, 1670 cm-', HRMS (FAB) no M+ observed.

Acidic Hydrolysis and Cyclization to Nitraramine. Trichloroacetic acid (0.5 mL) was added in one portion to an ice-cold solution of 16 (0.11 g, 0.22 mmol) in CH2Cl2 (1 mL). After 15 min at 0 °C the mixture was stirred at room temperature for 30 min and evaporated in vacuo (bath T < 25 °C). The oily residue (9 TFA salt) was dissolved in CCl4 by the addition of CD30D (2 drops): 1H NMR (CDCl3) δ 8.43 (bs, 2H), 6.87 (bt, 2H), 3.65 (m, 4H), 2.42 (m, 4H), 1.98 (m, 4H), 1.74 (m, 2H). This bis-iminium salt was not stable enough for further analysis and was evaporated directly after the acid-treatment and dissolved in a pH 7 phosphate buffer (20 mL). The resulting solution was refluxed under nitrogen during 20 h, made alkaline with excess solid carbonate, and extracted three times with ethyl acetate. The organic layer was dried (Na2SO4), and removal of the solvents gave a crude alkaloid mixture, which was pure enough for further analysis. Nitraramine in ethanol was treated with conc HCl. The solvents and the excess of HCl were removed by coevaporation with methanol, and the dihydrochloride was dissolved in ethanol and treated with a small excess of triethylamine. Evaporation and crystallization from CH2Cl2/ethyl acetate produced triethylamine hydrochloride, which was removed by filtration. The filtrate was evaporated to dryness and nitraramine hydrochloride was crystallized from a small amount of ethanol by precipitation with ethyl acetate. The free base was obtained in pure form by treatment of nitraramine hydrochloride with aqueous K2CO3 followed by ether extraction. 

Nitraramine (11): 1H NMR (CDCl3) δ 4.43 (m, 1H, H-7), 4.07 (d, J = 2.5 Hz, 1H-17), 3.54 (s, 1H, H-14), 3.11-3.07 (m, 2H-15), 3.07-3.04 (m, 1H, H-15eq), 2.79-2.71 (m, 1H-15ax), 2.69-2.61 (m, 1H-3ax), 2.19-2.14 (m, Jgem = 14.5 Hz, H-Beq), 2.19-2.16 (m, H-12), 1.85-1.45 (m, 14H); 13C NMR (CDCl3) δ 167.1, 153.2, 142.2, 130.1, 102.6, 82.65, 61.02, 45.77, 33.21, 28.06, 27.79, 24.30, 23.60, 22.22, 15.27; IR (CHC13) 1755, 1705, 1620 cm-', HRMS obs mass 283.1570.