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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 didehydroperipiperidine 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,3 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.

Remarkable is the racemic form in which nitraramine and several other Nitraria alkaloids occur in nature. In a recent publication5 Heathcock and co-workers synthesized petrosin, a racemic bisquinolizidone sponge alkaloid containing eight stereocenters. They nicely demonstrated that postbiosynthetic equilibrations 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 proposed1 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 synthesis8 of the indole alkaloids nitramine 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.9 Labeling studies8 in Lupine species have established dipiperidine 5 (tetrahydrobasine) as the biogenetic 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 unstable didehydroperipiperidine 410 as an intermediate has not been confirmed. An alternative ring opening of the saturated piperiderinering 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 didehydroperipiperidine 4 gives precursor 8, which is probably in equilibrium with the

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

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corresponding α,β-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. 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, a stable piperidine equivalent that can be converted efficiently into 4 via a reduction/deprotection sequence. 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 α,β-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 piperidone carbonyls 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-
to form 18 lowered the yield. Acid-catalyzed removal of the unsaturated lactam moiety followed by 1,2-reduction framework, and via cyclohexane ring-inversion of 1a-1ams.16 In our situation both lactam carbonyls of nitraramine and 1-epi-nitraramine. Shen, M. Y.; Zuanazzi, J. A.; Kan, C.; Quirion, J.-C.; Husson, H.-P.; Bick, I. R. C.; Alkaloids from Nitraria billardieri: Nut. billardieri; Nut. by NMR in the form of its TFA-salt; see Experimental Section.

Identification of nitraramine was possible by comparison of 1H and 13C NMR spectra with data from the literature.1718 Extensive 2D NMR-spectroscopy was performed on both nitraramine 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 vitro would require several redox-enzymes, was accomplished with N-Boc-piperidone as a diehydropiperidine 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 (0.303–0.075 mm). When ammonia-containing eluents were used, the silica gel was pretreated with this eluent.

5-2-Piperidiny1)-4,4'-didehydropiperidine Dihydro-
for purposes of this manuscript, we measured the chemical shifts of both chiral enantiomers in the NMR spectra. The coupling constants (J) were determined from 1D and 2D NMR experiments. The coupling constants were determined by comparing the spectra of the diastereomeric mixtures with those of the racemic compounds.

### Scheme 4

\[
\begin{align*}
9 & \overset{\text{H}_2\text{O}}{\underset{\text{pH7}}{\rightleftharpoons}} 8 \\
\text{Scheme 4} & \\
21: 1,3-diequatorial & \overset{\text{Cyclohexane ring-inversion}}{\iff} 21: 1,3-dialixal
\end{align*}
\]

Nitraramine

17 - 22%

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-piperidine ring 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 916 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 nitraramine as the only isomer. Spirocyclization of 8 completes the carbon-framework, and via cyclohexane ring-inversion of 21 the required 1,3-dialixal 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-nitraramine was recently isolated from Nitraria billardieri by Quirion17 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 Louanasmaa et al.18

### Scheme 5

\[
\begin{align*}
9 & \overset{\text{H}_2\text{O}}{\underset{\text{pH7}}{\rightleftharpoons}} 8 \\
\text{Scheme 5} & \\
7 & + & 4 & \iff 1,3-diequatorial
\end{align*}
\]

Identification of nitraramine was possible by comparison of 1H and 13C NMR spectra with data from the literature.1718 Extensive 2D NMR-spectroscopy was performed on both nitraramine 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 vitro would require several redox-enzymes, was accomplished with N-Boc-piperidone as a diehydropiperidine equivalent.
1 h and quenched with saturated NH₄Cl solution. Extractive 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 6 h. Aqueous workup and chromatographic separation (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 5.85 (t, J = 7 Hz, 1H), E-isomer: ¹H NMR (CDCl₃) δ 6.89 (m, J = 7.5 Hz, 4.1), δ (t, J = 5.4 Hz, 1H), 3.64–3.39 (m, 6H), 2.40 (m, 2H), 2.10 (t, J = 6.4 Hz, 2H), 1.80 (m, J = 5.4 Hz, 2H), 1.56 (m, 2H), 1.48 (s, 9H), 1.14 (t, J = 7.0 Hz, 3H), δ¹C NMR (CDCl₃) δ 167.1, 153.2, 142.2, 142.5, 130.0, 102.5, 82.65, 61.0, 45.77, 33.81, 28.06, 27.99, 26.30, 24.30, 22.22, 15.27; IR (CHCl₃) 1755, 1705, 1620 cm⁻¹; HRMS (FAB) obs mass 378.2180 (M⁺), calcd for C₂₃H₂₃NO₃Na 378.2383; obs mass 356.2400 (MH⁺), calcd for C₂₃H₂₂NO₂ 356.2503.

5-[N-(tert-Butyloxycarbonyl)-2-oxopiperidin-3-ylidenel]

pentanal (14). Acetal 13 (3.55 g, 10 mmol) was stirred with PPTS (0.12 g) in a mixture of water (5 mL) and acetone (150 mL) at 20 °C during 4 days. The reaction mixture was warmed up to 60 °C and distilled to (30 mL) solution (100 mL) to remove the catalyst. Extraction and drying yielded 14 (2.6 g, 100%), which was pure enough for further transformations. ¹H NMR (CDCl₃) δ 9.72 (t, J = 1.5 Hz, 1H), 6.86 (m, J = 5.5 Hz, 1H), 3.64 (m, 2H), 2.43 (m, 4H), 2.13 (q, J = 7.5 Hz, 2H), 1.88–1.71 (m, 4H), 1.49 (s, 9H); δ¹C NMR (CDCl₃) δ 201.8, 165.1, 153.1, 140.8, 130.9, 82.79, 45.80, 43.07, 27.99, 27.39, 24.33, 22.17, 20.37; IR (CHCl₃) 1755, 1750, 1700, 1620 cm⁻¹; HRMS (FAB) obs mass 281.1649, calcd for C₂₃H₂₂NO 281.1570.

1-[N-(tert-Butyloxycarbonyl)-2-oxopiperidin-3-yl]-5-
hydroxy-5-[N-(tert-Butyloxycarbonyl)-2-oxopiperidin-3-ylidenel]
pentane (15). A solution of the lithium enolate of 14 (0.072 g, 60%) as a mixture of isomers: ¹H NMR (CDCl₃) δ 6.91 (m, 1H), 5.84 (m, 1H), 3.64 (m, 2H), 2.43 (m, 4H), 2.13 (q, J = 7.5 Hz, 2H), 1.85–1.35 (m, 14H); ¹3C NMR (CDCl₃) δ 165.1, 153.2, 142.2, 130.1, 102.6, 97.89, 79.9, 73.32, 72.82; IR (CHCl₃) 3600–3200 (br) 1685, 1670 cm⁻¹; HRMS (FAB) no M⁺ observed.

Reduction of 15 with NaBH₄. Excess sodium borohydride was added to 15 (0.56 g, 2 mmol) in THF (15 mL) at -78 °C. After a total reaction time of 2.5 h the reaction was quenched with acetic acid (0.2 mL). Aqueous NaOH workup, extraction with ethyl acetate, and chromatography using CH₂Cl₂/ethyl acetate produced triethylamine hydrochloride, which was removed by filtration. The filtrate was evaporated to dryness and nitraramine hydrochloride 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 (1): ¹H NMR (CDCl₃) δ 4.43 (m, 1H, H-7), 4.07 (d, J = 2.5 Hz, 1H, H-17), 3.54 (s, 1H, H-11), 3.11–3.07 (m, 1H, H-17 eq), 3.07–3.04 (m, 1H, H-15 eq), 2.97–2.79 (m, 1H, H-15ax), 2.69–2.61 (m, 1H, H-3ax), 2.19–2.14 (m, J = 13.8 Hz, H-5eq), 2.01 (m, H-12), 1.9–1.45 (m, 7H, 1.44–1.32 (m, 3H, 2H-4, H-5eq), 1.26–1.23 (m, 1H, H-10), 1.17 (m, 1H), 1.11–1.01 (m, 2H, H-14ax, H-5ax), δ¹C NMR (CDCl₃) δ 82.30 (C-17), 75.95 (C-16), 65.54 (C-7), 60.50 (C-15), 49.35 (C-3), 39.84 (C-11), 39.79 (C-12), 33.37 (C-6), 30.55 (C-5), 28.50 (C-8), 25.22 (C-13), 24.12 (C-10), 21.94 (C-9), 15.40 (C-4) 14.61 (C-1), MS m/z (%): 162.1 (15), 160.1 (39), 158.1 (23), 156.1 (18), 152.1 (18), 150 (35); HRMS obs mass 248.1899, calcd for C₁₉H₂₁NO₂ 248.1857.

Nitraramine monohydrochloride: mp: 223–225 °C; ¹H NMR (D₂O) δ 4.34 (m, 1H, H-7), 4.27 (d, J = 2.5 Hz, 1H, H-17), 3.54 (s, 1H, H-11), 3.11–3.07 (m, 1H, H-17 eq), 3.07–3.04 (m, 1H, H-15 eq), 2.97–2.79 (m, 1H, H-15ax), 2.69–2.61 (m, 1H, H-3ax), 2.19–2.14 (m, J = 13.8 Hz, H-5eq), 2.01 (m, H-12), 1.9–1.45 (m, 7H, 1.44–1.32 (m, 3H, 2H-4, H-5eq), 1.26–1.23 (m, 1H, H-10), 1.17 (m, 1H), 1.11–1.01 (m, 2H, H-14ax, H-5ax), δ¹C NMR (CDCl₃) δ 82.30 (C-17), 75.95 (C-16), 65.54 (C-7), 60.50 (C-15), 49.35 (C-3), 39.84 (C-11), 39.79 (C-12), 33.37 (C-6), 30.55 (C-5), 28.50 (C-8), 25.22 (C-13), 24.12 (C-10), 21.94 (C-9), 15.40 (C-4) 14.61 (C-1), MS m/z (%): 162.1 (15), 160.1 (39), 158.1 (23), 156.1 (18), 152.1 (18), 150 (35); HRMS obs mass 248.1899, calcd for C₁₉H₂₁NO₂ 248.1857.

Supporting Information Available: Copies of the 1D and 2D NMR spectra of nitraramine 1 (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.