ORGANIC LETTERS

2005
Vol. 7, No. 12
2497−2499

Biomimetic One-Step Access to Nitraramine from Simple C_5 Units. Revision of the Previously Reported Structure of Epinitraramine to Nitraramine

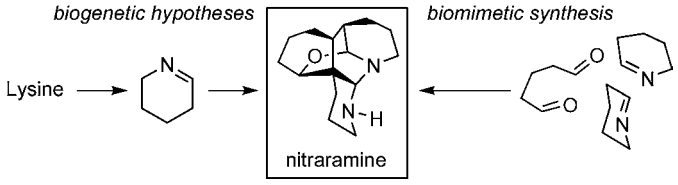
Edmond Gravel, Erwan Poupon,* and Reynald Hocquemiller

Laboratoire de Pharmacognosie associé au CNRS (UMR 8076, BioCIS), Centre d'Études Pharmaceutiques, Université Paris−Sud 11, 5, rue Jean-Baptispte Clément, 92290 Châtenay-Malabry, France

erwan.poupon@cep.u-psud.fr

Received April 18, 2005

ABSTRACT



A single biomimetic cascade sequence featuring intermolecular followed by intramolecular cyclizations allowed the biomimetic synthesis of nitraramine from simple C_5-lysine-derived metabolites. In the course of the study, the structure of epinitraramine was also revisited.

Piperidine alkaloids from *Nitraria* species (Zygophyllaceae) constitute a singular class of natural compounds both in terms of chemical diversity (e.g., many 3-spiropiperidines) and biosynthetic origin.[1] In fact, a particularly primitive lysine-based metabolism could account for the biogenesis of these alkaloids. Moreover, the fact that numerous *Nitraria* alkaloids are present as chiral but racemic forms in Nature strongly suggests a minimum enzymatic intervention, at least for the key diversity-generating cyclizations.[2] Herein, we report a totally biomimetic synthesis of nitraramine **1** in a one-pot multicomponent reaction, demonstrating the probably minimal role of enzymes.

Nitraramine **1**, a small but intricate structure, isolated from *Nitraria schoberi*[3] can be seen as a condensation of three C_5 units derived from lysine.[4] The formation of an endocyclic enamine **3** from lysine is a known step in the biosynthesis of many alkaloids such as in the *Lupinus* alkaloids. Dimerization of 2-piperideine **3** into tetrahydroanabasine **4** followed by opening and oxidation could lead to intermediate **5**, described as a pivotal key element in *Nitraria* alkaloid biogenesis.[1,5] From **5**, the formation of **1** can be explained through a series of virtually equilibrated reactions essentially consisting of imine/enamine tautomerism, Michael/retro-Michael and Mannich/retro-Mannich reactions, and hydration/dehydration. In the last step, following a ring inversion, a nucleophilic attack of the hydroxyl and a last aza-Mannich reaction onto the remaining iminium system afford **1**. In this

(1) Wanner, M. J.; Koomen, G.-J. In *Studies in Natural Products Chemistry: Stereoselectivity in Synthesis and Biosynthesis of Lupine and Nitraria Alkaloids*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1994; Vol. 14, pp 731−768 and references therein.

(2) Wanner, M. J.; Koomen, G.-J. *Pure Appl. Chem.* **1996**, *68*, 2051−2056.

(3) Tashkodzhaev, B.; Ibragimov, A. A.; Yunusov, S. Y. *Khim. Prir. Soedin.* **1985**, 692−698; *Chem. Nat. Compd. (Engl. Transl.)* **1985**, 649−655.

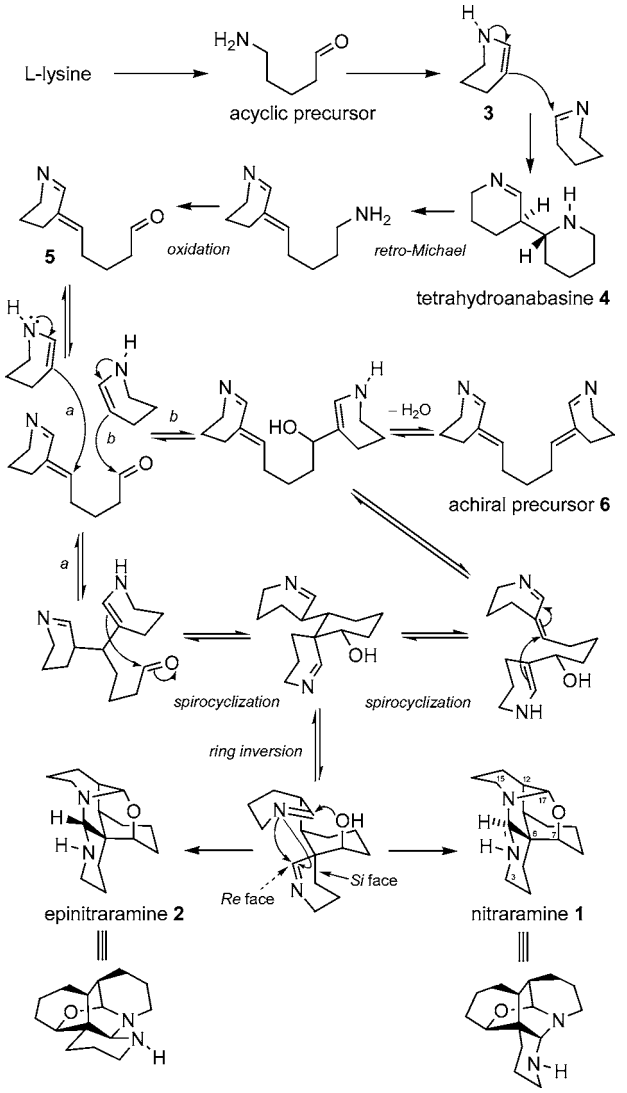
(4) Wanner, M. J.; Koomen, G.-J. *J. Org. Chem.* **1995**, *60*, 5634−5637.

(5) François, D.; Lallemand, M.-C.; Selkti, M.; Tomas, A.; Kunesch, N.; Husson, H.-P. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 104−105; *Angew. Chem.* **1998**, *110*, 112−114.

10.1021/ol050849q CCC: $30.25
Published on Web 05/17/2005

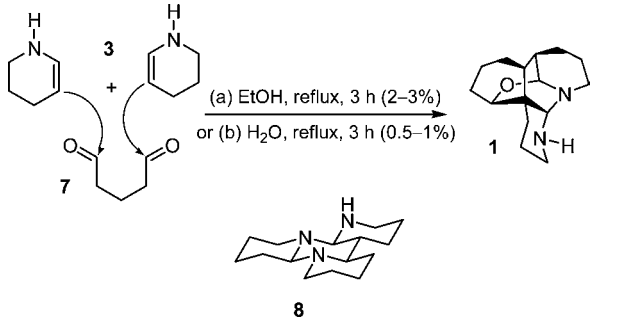
context, formation of 1-epinitraramine, which was assigned the structure **2**,[6] in conjunction with **1**, has been explained by a likely attack on the *Si*-face or the *Re*-face of the iminium during the aminal formation to produce **1** and **2**, respectively.

Scheme 1. Proposed Biogenetic Pathway to Nitraramine **1**



Plausible achiral intermediate **6** was targeted by a stepwise approach by Koomen and colleague in their beautiful, pioneering synthesis of **1** (seven steps, ~0.5% yield).[4] In connection with the proposed biosynthetic pathway, we reasoned that intermediate **5** was the simplest precursor that could lead to **1** in a biomimetic cascade sequence. The laboratory assembly of **5** should be possible by in situ reaction of a molecule of 2-piperideine **3** with 1 equiv of glutaraldehyde **7** (which can also be considered as a lysine C_5 unit metabolite); the intervention of a second molecule of **3** is then necessary to furnish **1** without any further oxidation/reduction steps. Once we had chosen the correct starting precursors in terms of oxidation state, the stage was set for condensation studies: **3**[7] and **7** (2:1 ratio) were reacted in boiling ethanol for 3 h.

Scheme 2. Biomimetic Synthesis of **1**



The purification step was feared, as the TLC profile of the crude reaction mixture was complex. The range of polarity was known from literature,[8] and NMR-guided fractionations (especially the location of the H-1 proton) enabled the exact location of **1** to be determined on TLC (CH_2Cl_2/MeOH 9:1, R_f = 0.35). Although unexpected, the preparative purification outcome finally appeared to be quite trivial. After optimization, pure **1** was obtained by two consecutive chromatography purifications on basic alumina. In ideal biomimetic conditions, the reaction should be conductible in water; the same condensation protocol was therefore tried in boiling water. Isolation of **1** was possible but in lower yield (0.5−1 vs 2−3% in EtOH), due to an important loss of material in water during workup. From the standpoint of efficiency and despite low but reproducible yields (2−3%), a total of five new bonds (including three carbon−carbon bonds) and six chiral centers (including one quaternary spiro-carbon) are formed in a single vessel.[9] Once the target molecule was clearly identified in the crude mixture, we at first sight did not give attention to the multiple other compounds formed during this kind of "biomimetic combinatorial process". Among major compounds formed

(6) Epinitraramine was characterized from *Nitraria billardieri*; see: Shen, M. Y.; Zuanazzi, J. A.; Kan, C.; Quirion, J.-C.; Husson, H.-P.; Bick, I. R. C. *Nat. Prod. Lett.* **1995**, *6*, 119−125.

(7) Formed in situ from its crystalline trimeric form. For preparation from piperidine, see inter alia: de Kimpe, N.; Stevens, C. *J. Org. Chem.* **1993**, *58*, 2904−2906.

(8) Chromatography column conditions in ref 4 (CH_2Cl_2/MeOH/NH_4OH 90:10:1).

(9) **Synthesis of 1 from 3.** 2-Piperideine **3** (in its trimeric form, 5 g, 0.06 mol) was dissolved in ethanol (500 mL). Immediately after dissolution, glutaraldehyde (0.5 equiv, 0.03 mol, 12 mL of a 25% aqueous solution) was added. The mixture was stirred under reflux for 3 h and then concentrated under reduced pressure. Purification of the crude mixture was performed on two consecutive alumina columns (CH_2Cl_2/MeOH 99:1, 98:2, 97:3, and finally 96:4) and monitored by TLC on silica gel (CH_2Cl_2/MeOH 9:1) to furnish pure nitraramine **1** (165−225 mg, 2−3%). **1**: colorless crystals; R_f = 0.35 (silica gel, CH_2Cl_2/MeOH 9:1); IR (film, $CHCl_3$) ν_{max} = 2928, 1635, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ filtered through activated alumina) δ 4.43 (1H, m, H-7), 4.06 (1H, d, J = 2 Hz, H-17), 3.36 (1H, s, H-1), 3.10 (2H, m, H-3_{eq}, H-15_{eq}), 2.68 (2H, m, H-15_{ax}, H-3_{ax}), 2.17−2.13 (1H, m, J_{gem} = 13.7 Hz, H-5_{eq}), 2.00 (1H, m, H-12), 1.87−1.38 (10H, m), 1.26−1.22 (1H, m, H-10_{ax}), 1.16 (1H, m, H-11), 1.09−1.00 ppm (2H, m, H-14_{ax}, H-5_{ax}); ^{13}C NMR (100 MHz, $CDCl_3$ filtered through activated alumina) δ 82.2 (C-17), 75.9 (C-1), 66.5 (C-7), 50.5 (C-15), 45.1 (C-3), 38.8 (C-11), 38.0 (C-12), 32.4 (C-6), 30.4 (C-5), 28.4 (C-8), 25.2 (C-13), 24.1 (C-10), 21.6 (C-4), 15.3 (C-9), 14.6 ppm (C-14); HRMS (ES) calcd for $C_{15}H_{24}N_2OH^+$ [M + H^+] 249.1967, found 249.1969.

during the reaction, stable trimer **8** has been identified. Careful studies are currently underway to elucidate the structure of other molecules that were formed in smaller quantities.[10] The other potential problem was the apparent absence of 1-epinitraramine **2** in the course of our reaction. As already pointed out, nitraramine appeared to be remarkably stable,[4] even if all chiral centers could, in principle, be scrambled via retro-Michael and retro-Mannich reactions. We quickly realized, when comparing compiled NMR data,[11] that none of the ^{1}H NMR spectra were totally superimposable. Furthermore, spectra of our synthetic **1** differed to some extent, even when coming from the same batch in bulky $CDCl_3$. A simple chemical shift versus concentration study clearly demonstrated that the compound previously described as epinitraramine **2** was in fact diluted nitraramine **1** in the NMR tube (Figure 1 shows the most significantly variable

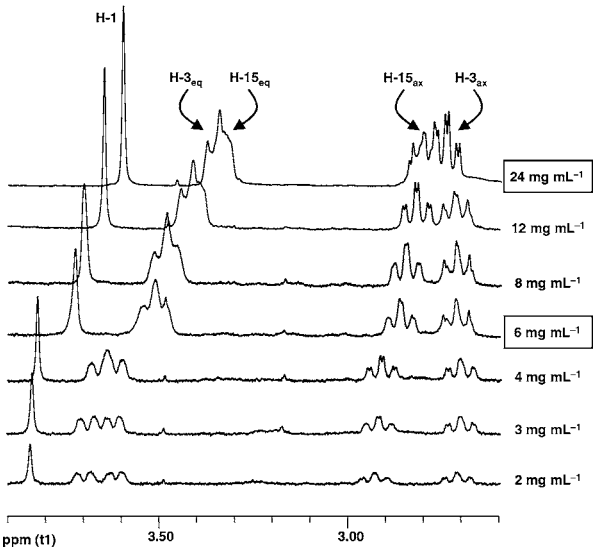


Figure 1. Concentration influence on chemical shifts of **1** (400 MHz, 25 °C).

area of the spectrum, under different concentration conditions). As a matter of fact, the observed spectrum for a solution of about 6 mg mL^{-1} is equivalent to the one attributed to epinitraramine,[6] and the spectrum observed for a solution of about 24 mg mL^{-1} is equivalent to the one attributed to nitraramine by Quirion and colleagues.[6] We also observed that when diluted samples were concentrated again, the observed ^{1}H NMR spectrum was that of nitraramine **1**. Each spectrum in Figure 1 probably reflects a different degree of protonation, showing a different average of the two chemical forms. In fact, according to the ^{1}H NMR chemical shift time scale, protonated/deprotonated states are considered in fast exchange giving rise to a weighted averaged spectrum of extreme values (i.e., δ (protonated) and δ (free base)). When, prior to utilization, traces of acid in $CDCl_3$ were removed by filtration over basic Al_2O_3 and **1** was treated with K_2CO_3, no difference was noticeable by ^{1}H NMR analysis, regardless of the concentration, all spectra corresponding to what was reported by Koomen and colleague as **1**.[4] Therefore, epinitraramine **2**, which was only characterized upon NMR analysis, is likely to be an experimental artifact. In terms of chemical assembly, the cascade involved in the formation of **1** appears therefore to be highly stereoselective.

The low yield of **1** reflects the multiple biomimetic coupling possibilities of lysine-derived precursors **3** and **7** but argues for the incontestable spontaneous formation of **1** from fundamental C_5 units presumably derived from lysine. Bearing in mind that the poor yield of our synthesis is a significant drawback, one can add that the ease and reliability of the reaction conditions from inexpensive reagents permit the preparation of pure **1** in a time scale of a few hours. Therefore, the described total synthesis of **1** competes largely with scarce extraction from natural sources (which are present only in restricted areas) and with the previous total synthesis. Enough material has been prepared for needed biological screening of this intriguing, complex small molecule. Furthermore, total synthesis unexpectedly led to a structural reassignment of epinitraramine **2**.[12] Finally, the chemistry of simple C_5-lysine-type molecules[13] presented in this paper offers further opportunities toward biomimetic synthesis[14] and molecular self-assembly[15] of complex skeletons, and other examples in this area are currently under investigation in our group.

Acknowledgment. We thank Prof. Dr. Henri-Philippe Husson and Dr. Nicole Kunesch for fruitful discussions and advice. We also thank Jean-Christophe Jullian for NMR spectroscopic assistance.

OL050849Q

(10) Other *Nitraria* alkaloid skeletons are likely to be assembled in the reaction, as well as potentially new natural-product-like scaffolds; complete details will be discussed in a full account of this work.

(11) NMR data from refs 4 and 7. We thank Prof. Dr. Henri-Philippe Husson for providing us with NMR spectra of **1** and **2**.

(12) For a recent review article concerning misassigned natural products, see: Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012−1044.

(13) For recent examples of cascade and multicomponent reactions involving successive condensations of simple building blocks to assemble complex structures, see inter alia: (a) Hourcade, S.; Ferdenzi, A.; Retailleau, P. Mons, S.; Marazano, C. *Eur. J. Org. Chem.* **2005**, 1302−1310 and references therein. (b) Amorde, S. M.; Judd, A. S.; Martin, S. F. *Org. Lett.* **2005**, *7*, 2031−2033.

(14) For a recent review concerning biomimetic synthesis, see inter alia: de la Torre, M. C.; Sierra, M. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 160−181. For a review on biomimetic synthesis of alkaloids, see inter alia: Scholz, U.; Winterfeldt, E. *Nat. Prod. Rep.* **2000**, *17*, 349−366.

(15) For comments on molecular self-construction, see: Sorensen, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 3225−3228.