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# Biomimetic Synthesis of Tangutorine Following New Biogenetic Proposals†

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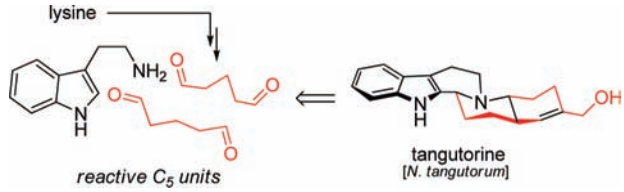
## ABSTRACT




**Following new biosynthetic proposals, an expeditious synthesis of tangutorine, an indolic alkaloid from *Nitraria tangutorum* has been achieved in three steps from simple $C_5$ lysine-derived units. The work also includes further insights into the biosynthesis of *Nitraria* alkaloids.**

Tangutorine **1** was isolated by C.-T. Che in 1999 from the leaves of *Nitraria tangutorum* (Zygophylaceae/Nitrariaceae) collected in China.[1] The structure, ascertained by X-ray analysis, features a benz[*f*]-indolo[2,3-*a*]quinolizidine unit unique among natural products. Another interesting issue for us is the fact that **1** was isolated as a racemate. Tangutorine was recently shown to have interesting effects on the regulation of cell cycle and cellular morphology on HT29 human colon cancer cells. A detailed study demonstrated an induction of protein p21 and an inhibition of topoisomerase II expressions in these cells.[2] Since its discovery, the unusual structure of **1** (Figure 1) stimulated synthetic work beginning with the first total synthesis by R. Jokela and collaborators in 2001,[3] followed by the work of R. P. Hsung[4] and T.-L. Ho.[5] A formal synthesis[6] and synthetic approaches[7] have also been disclosed. Herein we report an expeditious synthesis of **1** based upon our own biosynthetic analysis.

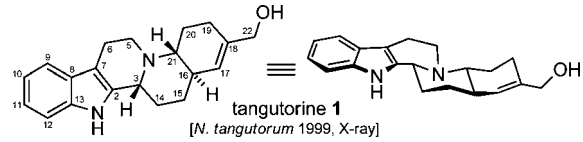



**Figure 1.** Structure of tangutorine **1**.

Relying on the similarity with yohimbane alkaloids, Jokela and collaborators proposed a possible indolomonoterpenic biosynthetic origin for **1** (Scheme 1). Two disconnection/connection sequences are needed to explain the conversion of the yohimbane skeleton into the tangutorine skeleton, that is, first C-15/C-16 and C-16 to C-21, then C-21/N-4 and C-19 to N-4.[8] Several remarks have to be formulated concerning this proposal. First, whereas the C-15/C-16 disconnection is

† Dedicated to the memory of Christian Marazano.

(1) Duan, J.-A.; Williams, I. D.; Che, C.-T.; Zhou, R.-H.; Zhao, S.-X. *Tetrahedron Lett.* **1999**, *40*, 2593–2596.

(2) Liu, B. P. L.; Chong, E. Y. Y.; Cheung, F. W. K.; Duan, J.-A.; Che, C.-T.; Liu, W. K. *Biochem. Pharmacol.* **2005**, *70*, 287–299.

(3) (a) Putkonen, T.; Tolvanen, A.; Jokela, R. *Tetrahedron Lett.* **2001**, *42*, 6593–6594. (b) Putkonen, T.; Tolvanen, A.; Jokela, R.; Caccamese, S.; Parinello, N. *Tetrahedron* **2003**, *59*, 8589–8595.

(4) Luo, S.; Zificsak, C. A.; Hsung, R. P. *Org. Lett.* **2003**, *5*, 4709–4712.

(5) Ho, T.-L.; Chen, C.-K. *Helv. Chim. Acta* **2006**, *89*, 122–126.

(6) Luo, S.; Zhao, J.; Zhai, H. *J. Org. Chem.* **2004**, *69*, 4548–4550.

(7) Wilkinson, J. A.; Ardes-Guisot, N.; Ducki, S.; Leonard, J. *Tetrahedron Lett.* **2005**, *46*, 8053–8056.

(8) Using the classical "biogenetic" numbering proposed by Le Men and Taylor which has the advantage of showing the structural homogeneity within the apparent diversity of these alkaloids.

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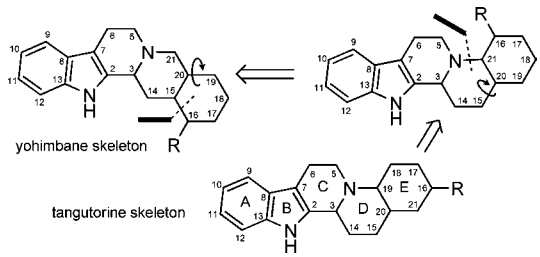
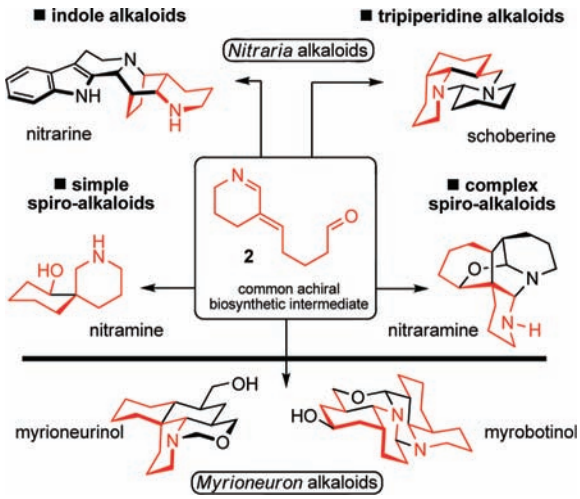
**Scheme 1.** Initial Biogenetic Proposal for Tangutorine




the fundamental initial step in the biosynthesis of rearranged monoterpenoid indole alkloids (e.g., of the ibogane or aspidospermane types), the other postulated steps are far from being trivial. If one considers a biosynthesis from yohimbine (which appears to be the closest natural compound related to **1**), the relative stereochemistry at C-3 has to be inversed. This scheme also does not explain why **1** is extracted as a racemate in nature which would implicate a total racemization of all stereogenic centers in yohimbine. Finally, a close look at all publications related to the isolation of "*Nitraria* alkaloids" revealed neither any yohimbane type precursors or analogs nor any terpenoid alkaloids in general in the *Nitraria* genus.[9]

Alkaloids from the *Nitraria* genus (Nitrariaceae) which comprise a range of diverse skeletons are characterized by a common biosynthetic origin, namely a particular lysine-derived metabolism. We[10] and other pioneering groups[11] have shown that a common biosynthetic pathway can be postulated. Starting from pivotal achiral precursor **2**, access to all groups of *Nitraria* alkaloids can be envisioned as shown in Scheme 2 with the examples of simple (e.g., nitramine)

**Scheme 2.** Common Intermediate in the Biosynthesis of *Nitraria* and *Myrioneuron* Alkaloids




or complex (e.g., nitraramine) spiroalkaloids, indole alkaloids (e.g., nitrarine) or tripiperidinic molecules (e.g., schoberine). Interestingly, recently described alkaloids isolated from the *Myrioneuron* genus (Rubiaceae[12]) such as myrioneurinol[13] or myrobotinol[14] also seem to be derived from **2**.

In fact, **2** can be seen as a lysine-derived intermediate via endocyclic enamine **3** and then tetrahydroanabasine **4** as depicted in Scheme 3. The attack of a 2-piperidine unit on a molecule of glutaraldehyde **5** followed by a dehydration step could also be a potential path for the formation of **2**. Based on this, we logically turned our intention to finding a plausible biosynthetic scheme that could also secure a straightforward retrosynthetic scheme to tangutorine **1**.

In fact, a direct precursor of tangutorine such as **6** can be seen as the product of hydrolysis followed by oxidative deamination of achiral precursor **2**. An intramolecular aldolisation/crotonization could generate a cyclohexadiene **7** (the E ring of **1**). The latter could undergo a condensation with tryptamine **8** featuring a Pictet-Spengler reaction and a 1,6-Michael addition to form the D ring of **1**. Finally, tangutorine might be biosynthesized after reduction of the remaining aldehyde of intermediate **9** into a primary alcohol (Scheme 4). Alternatively, **7** may formally be considered as arising directly from two molecules of dialdehyde **5** by aldolisation/crotonization.

(9) With the exception of nitraraine which structure is questioned. An extensive survey on *Nitraria* alkaloids will soon be disclosed elsewhere as a review article.

(10) (a) Gravel, E.; Poupon, E.; Hocquemiller, R. *Org. Lett.* **2005**, *7*, 2497–2499. (b) Gravel, E.; Poupon, E.; Hocquemiller, R. *Tetrahedron* **2006**, *62*, 5248–5253.

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(12) This is of particular interest in terms of chemotaxonomy. The two families are not related to one another.

(13) Pham, V. C.; Jossang, A.; Sévenet, T.; Nguyen, V. H.; Bodo, B. *Tetrahedron* **2007**, *63*, 11244–11249.

(14) Pham, V. C.; Jossang, A.; Sévenet, T.; Nguyen, V. H.; Bodo, B. *J. Org. Chem.* **2007**, *72*, 9826–9829.

(15) Compound **10** was reported before, it was characterized, at this time, as its perfluorobenzylhydroxylamine adduct. See: Tashima, T.; Imai, M.; Kuroda, Y.; Yagi, S.; Nakagawa, T. *J. Org. Chem.* **1991**, *56*, 694–697. No yield of the reaction was provided.

(16) The yield is obviously low and could easily constitute a major drawback of the synthesis, but experimentally speaking, glutaraldehyde **5** is a cheap reagent and **10** is the most apolar compound of the reaction based on silica gel TLC profile which renders its purification by chomatography easy. In practise, 15 g of **5** give 3−4 g of **10** in a reproducible manner. We will give a full account of the other compounds isolated in due course, but **5**, as suspected, mainly gives complex polymers.

(17) This intermediate is putative and the pathway depicted is one among others. The treatment of **10** in the same conditions but without tryptamine did not allow the isolation of **7** (**10** remained mostly unchanged and was partly converted into *O*-acetyl-**10**).

(18) Which could be isolated as a single diastereomer by recrystallization of a trifluoroacetate salt precipitated from $H_2O/CH_3CN$ (8:2).

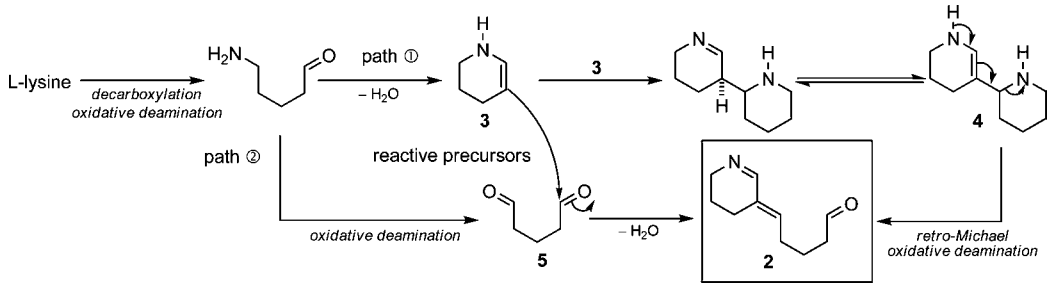
(19) See Supporting Information. Data were in agreement with the extensive analysis reported in R. P. Hsung's work (ref 4).

(20) Analytical conditions, see Supporting Information, could not be transposed to preparative HPLC due to solubility/precipitation issues.

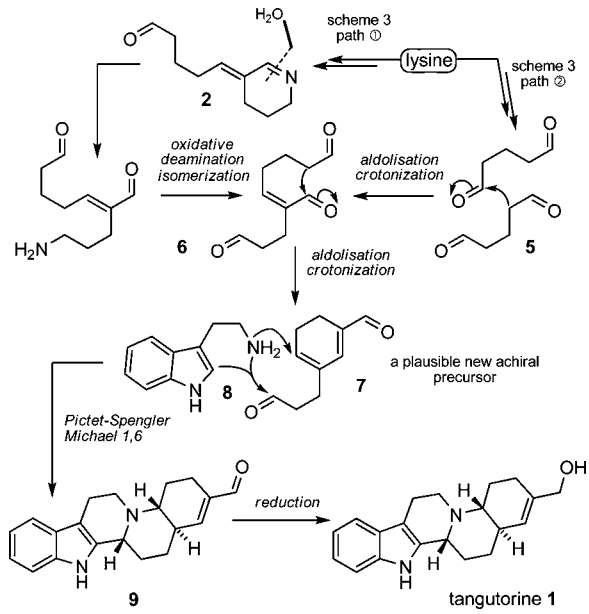
(21) As exemplified, here, by the fact that **1** is isolated as an optically inactive compound as it is the case for a large majority of *Nitraria* alkaloids with the exception of simple spiro-alkaloids such as nitramine. For a recent discussion on the impact of spontaneity in the biosynthesis of natural products, see: Poupon, E.; Gravel, E. *Eur. J. Org. Chem.* **2008**, 27–42.

(22) Tulyaganov, T. S.; Ibragimov, A. A.; Yunusov, S. Y.; Yu, *Chem. Nat. Compd.* **1982**, *18*, 604–606.

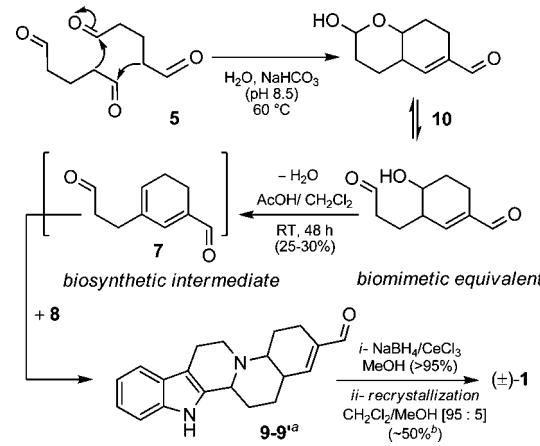
**Scheme 3.** Two Putative Pathways for the Biosynthesis of Key-Intermediate **2**




**Scheme 4.** Alternative Biosynthetic Pathway According to *Nitraria* Metabolism

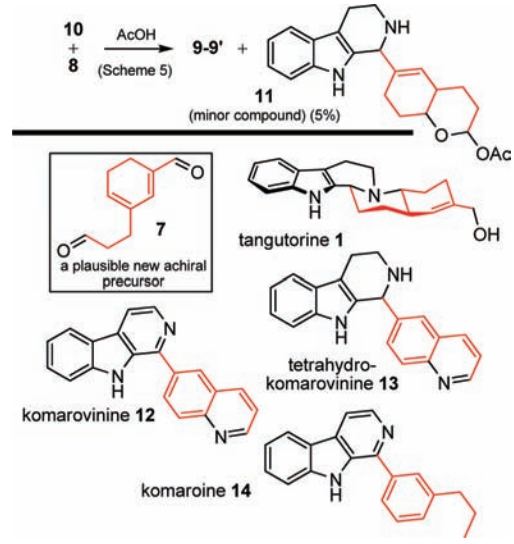



**Scheme 5.** Biomimetic Synthesis of Tangutorine




[a] As a mixture (75/25) of diastereomers. [b] Starting from **9**-**9′**.

**Scheme 6.** Putative New Achiral Precursor Based on the Isolation of a Minor Compound




On the basis of this new biosynthetic hypothesis, we began investigating a biomimetic access to **1** (Scheme 5). As a simple reactive $C_5$ surrogate of lysine we chose glutaraldehyde **5**. Treatment of **5** in basic conditions ($NaHCO_3$ aqueous solution at 60 °C) afforded compound **10** after careful purification by chromatography.[15,16] Compound **10**, obtained as an inextricable mixture of diastereomers of both aldehyde/acetal forms, was welcome as it is an equivalent of achiral cyclic precursor **7** as postulated in Scheme 4. It was then time to study the reactivity of **10** and its behavior in reaction with tryptamine **8**. Among many attempts, a 3:1 mixture of dichloromethane and acetic acid was suitably adapted to the condensation reaction. Compound **9** was obtained albeit in moderate yield upon reaction of **10** and **8** at room temperature. This unexpected straightforward outcome of the reaction can be rationalized by the *in situ* formation of achiral biosynthetic intermediate **7**.[17] This direct precursor of tangutorine **1** was obtained as a 75: 25 mixture of diastereomers (**9** and **9′**) which were unfortunately unseparable at this stage. A final reduction of **9**−**9′** using $NaBH_4$−$CeCl_3$ gave a mixture of diastereomers from which tangutorine **1** was obtained after recrystallization from $CH_2Cl_2$/MeOH (95: 5). Our NMR data and those reported for natural and

synthetic **1** were in agreement despite, as already observed in the literature, minor differences depending on the solvents and the inherent difficulties to solubilize **1** in NMR solvents. Relative configuration could be deduced from detailed analysis of **9**[18] and **1**.[19] This facile access to **1** was clouded with difficulties to isolate the minor diastereomer and therefore to characterize it: preparative HPLC purifications did not give satisfactory results.[20]

We have achieved a rapid synthesis of tangutorine that demonstrates the power of biomimetic synthesis when, in nature, assembly of complex architectures visibly arises with minimal enzyme intervention.[21]

We also further demonstrated a unified biosynthetic scenario from lysine-derived tetrahydroanabasine **4**. In addition, from the condensation reaction of **10** with tryptamine **8**, it was possible to isolate a minor compound which structure was deduced as being **11**. It results from a Pictet-Spengler reaction implying the $\alpha,\beta$-unsaturated aldehyde function of **10**. It is striking that **11** displays a skeleton that is reminiscent of that of natural substances such as komarovinine **12**,[22] tetrahydrokomarovinine **13**[23] or komaroine **14**[24] (Scheme 6) previously isolated from *Nitraria* species. Therefore, besides previously accepted achiral precursor **2** (Scheme 2), we can propose **7** as a novel achiral precursor for alkaloids such as **1**, **12**−**14**.

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**Supporting Information Available:** Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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