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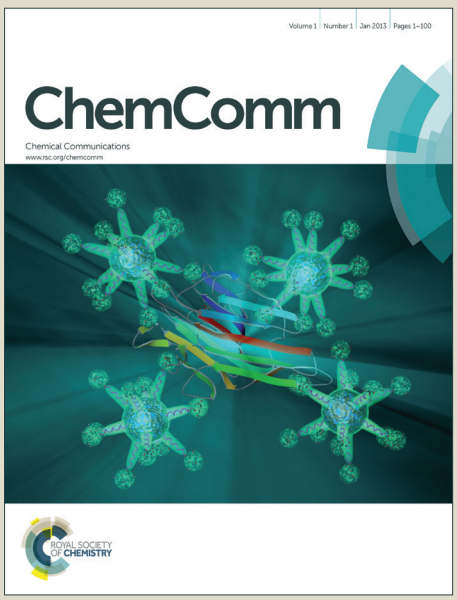





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# Biomimetic synthesis of Tramadol

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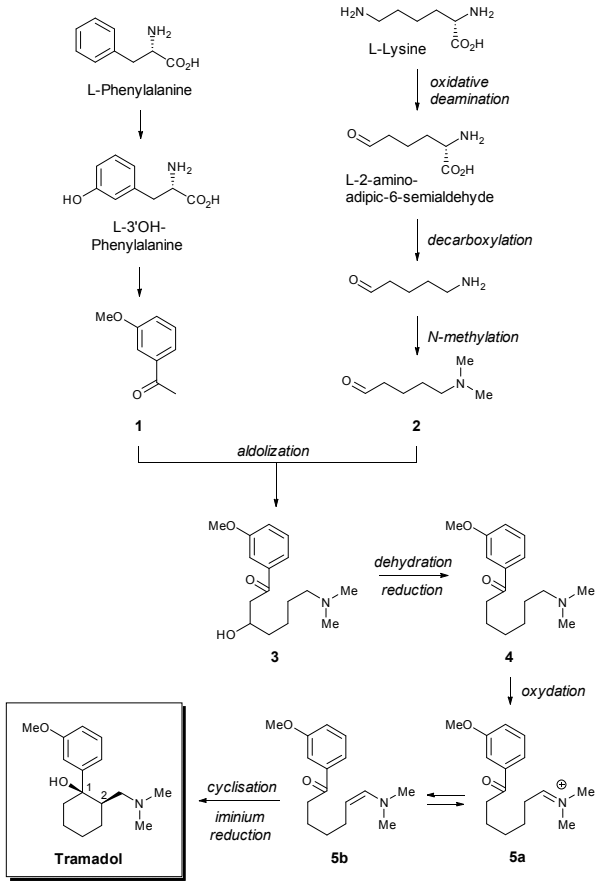
**In 2013, we reported that the root bark extract of *Nauclea latifolia* Sm. (Rubiaceae) collected in a biosphere reserve in the north of Cameroun contains large quantities (0.4% w/w) of (±)-(*1R,2R*)-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl)- Tramadol has recently been isolated from the roots and bark of *Nauclea latifolia*. A plausible biosynthetic pathway has been proposed and the product-precursor relationship probed by $^{13}$C position-specific isotope analysis. Further exploring this pathway, we demonstrate that a key step of the proposed pathway can be achieved in mild conditions that mimic in vivo catalysis.**

cyclohexanol.[1] The compound was isolated by a blind bioassay-guided search and the structure rigorously established by spectroscopic and X-ray diffraction analyses. It proved to be structurally identical to the commercialized analgesic, Tramadol, previously only known as a synthetic pharmaceutical used worldwide since 1977[2] as a painkiller by acting as a weak μ-opioid receptor agonist.[3] This discovery was highly publicized and covered by the major worldwide press.[4] Recently, Kusari et al.[5] reported the isolation of trace amounts of Tramadol (<0.00002% w/w) and metabolites thereof from *N. latifolia* and soils collected in the north of Cameroun and suggested a possible anthropogenic contamination through cattle and human overconsumption of Tramadol. However, this could equally plausibly be due to simian dispersal following feeding on *N. latifolia*, and the high concentration found in different samples of *N. latifolia*, as reported in the original work of Boumendjel et al.[1], cannot be explained by anthropogenic contamination. In addition, the samples were collected in the preserved Benoué biosphere reserve, in which human activities, as well as cattle pasturing, are prohibited.




**Scheme 1** Key intermediates and steps in the proposed biosynthesis of Tramadol, partially corroborated by a recent position-specific isotope analysis.[8]

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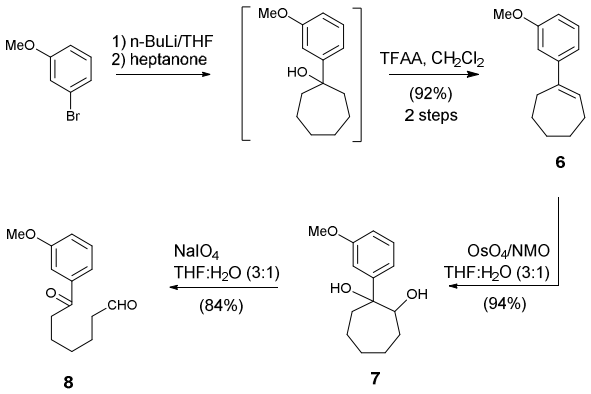
In order to provide evidence to support the probable natural origin of tramadol from *N. latifolia*, we have adopted two approaches to provide a plausible biosynthetic route. As it contains a basic amine, natural Tramadol can be classified as an alkaloid, for which the biosynthesis frequently involves one or more amino acid precursors, notably L-lysine, L-arginine, L-tyrosine, L-phenylalanine or L-tryptophan.[6] In the case of Tramadol, as outlined in Scheme 1 the proposed pathway invokes the condensation of 3'-methoxyacetophenone **1** with *N*,*N*-dimethyl-5-aminopentanal **2** to afford intermediate **3** which, upon reduction, provides amino ketone **4**. The latter would undergo an oxidation step to afford iminium **5a** in equilibrium with enamine **5b**. Finally, the key step is the cyclisation of enamine **5b** followed by the reduction of the resulting iminium to afford Tramadol.

In our first approach, the logical involvement of L-phenylalanine, L-lysine, **1** and **2** in the putative biosynthetic pathway of Tramadol has been detailed and related to known biosynthetic compounds,[7] a relationship partially corroborated by the analysis of the position-specific isotope.[8] In addition, L-lysine is known to initiate, through decarboxylation and oxidative deamination steps, the biosynthesis of 5-aminopentanal which leads to indolo[2,3-a]quinolizidine derivatives,[9] a class of natural compounds widely present in *N. latifolia* (*e.g.* angustine, nauclefine, naucletine,[10] strictosamide, naucleamides[11] and latifoliamides.[12] Furthermore, naturally occurring alkaloids sharing certain characteristics of Tramadol, such as the presence of an uncommon 3-methoxyphenyl substituent, have been reported.[13]

Two key steps of the proposed biosynthetic pathway can be identified: *(i)* the aldolization to form the full open carbon structure **3** and *(ii)* the cyclisation/reduction sequence by which enamine **5b** is converted to Tramadol. In our second approach, reported in this communication, we present evidence that *(ii)* can occur under mild biomimetic conditions. It should be noted that the cyclisation reaction could be enzymatically-catalyzed or not, as found in other alkaloid pathways.[14]

Herein, we focused our efforts on the synthesis of the iminium **5a** and its conversion via enamine **5b** to Tramadol. Iminium **5a** could be obtained from dicarbonyl **8**. This intermediate was prepared through a series of four straightforward steps (Scheme 2). Starting from 3-bromomethoxybenzene and cycloheptanone in presence of *n*-BuLi, a lithium-halogen exchange allows the nucleophilic addition, followed by a subsequent dehydration step. The resulting compound **6** was then submitted to a classical $OsO_4$/$NaIO_4$ treatment, leading to the oxidative cleavage of the alkene group to afford compound **8**, with an overall yield of 73%.

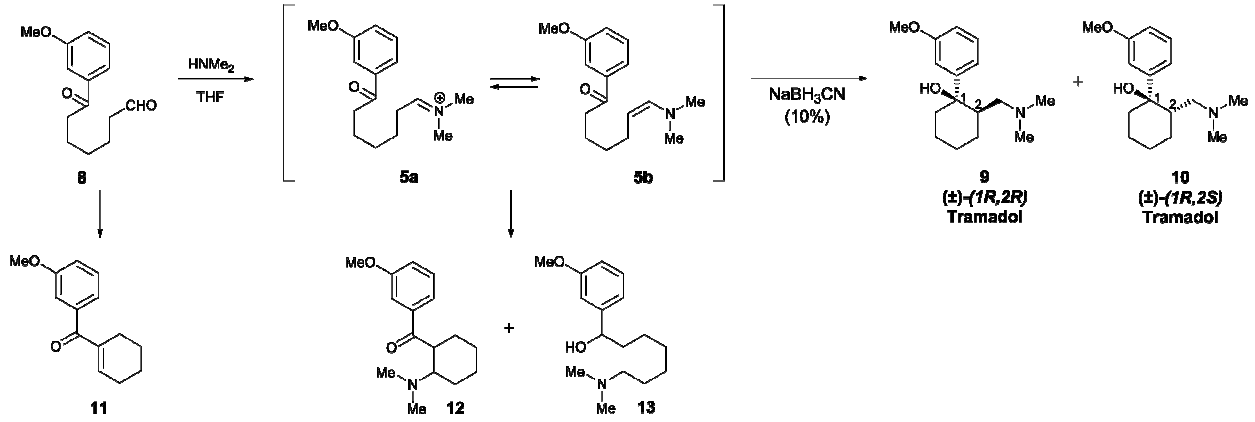



**Scheme 2** Preparation of dicarbonyl **8**, precursor of the key intermediates **5a**/**5b**.

Having the dicarbonyl derivative **8** in hand, we proceeded with its conversion to iminium **5a** by treatment with dimethylamine (Scheme 3). Despite several attempts, the isolation of iminium **5a** or enamine **5b** in acceptable yields was problematic and irreproducible. As an alternative, we attempted a one-pot conversion of **8** to Tramadol without isolation of **5a** and **5b**. Upon treatment of **8** with dimethylamine followed by an *in situ* reduction with $NaBH_3CN$ (or $NaBH_4$), the formation of Tramadol as a mixture of two diastereoisomers (**9** and **10**) in 10% yield and of at least three new compounds (**11**, **12** and **13**) was evidenced by UHPLC-TOF-MS and $^1$H NMR analyses. The formation of the α,β-unsaturated ketone **11** could readily originate from an intramolecular aldolization/crotonization of **8**, promoted by the basic dimethylamine. The aminoketone **12** could be formed *via* an intramolecular Mannich reaction involving the enolization ability of the ketone function of intermediate **5a**. Finally, the aminol **13** is probably formed from the double reduction of **5b**.

The formation of Tramadol proceeds *via* the iminium **5a** and subsequent isomerization to enamine **5b.** An intramolecular addition of the enamine onto the carbonyl according to a 6-enolexo Hajos-Parrish-Eder-Sauer-Wiechert[15] mechanism will afford an iminium intermediate, which upon reduction provides Tramadol **9** and its diastereoisomer **10**.

Tramadol was then purified by semi-preparative HPLC,




**Scheme 3** Conversion of **8** to Tramadol and by-products (**11**, **12** and **13**).

scaling-up analytical HPLC conditions using a gradient transfer method.[16] NMR analysis revealed a mixture of (±)-(*1R,2R*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol **9** and (±)-(*1R,2S*)-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl)-cyclohexanol **10** in a 7/3 ratio. The spectroscopic data of an authentic synthetic sample of Tramadol were identical in all aspects with those of Tramadol **9** obtained by the biomimetic route. The observed diastereoselectivity in favor of the (±)-(*1R,2R*)-isomer (**9**) may be due to steric hindrance caused by the methoxyphenyl moiety, differentiating the face by which the approaching nucleophile can access the diastereotopic faces of the carbonyl group.

Thus, under mild chemical conditions, we have demonstrated that a key intermediate of Tramadol biosynthesis **5b** can be formed *in situ* and can be converted to Tramadol without enzymatic catalysis. The involvement of a non-enzymatic ring closure readily explains the occurrence of a natural racemate, formed as a result of either *re*- or *si*- attack of the electron on the C1 position. The finding that the formation of the (±)-(*1R,2S*)-isomers is less favored and that these do not occur in the *N. latifolia* extract, adds weight to the natural origin of this compound.

As with the recent report regarding the position specific isotope analysis studies[8] this work gives further guidelines as to how to conduct investigations of the biosynthesis of this unusual compound by using labeling techniques.

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