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Total Synthesis of Incarvilleatone and Incarviditone: Insight into Their Biosynthetic Pathways and Structure Determination

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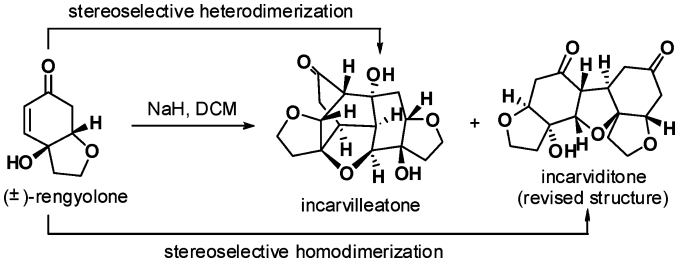
ABSTRACT



A concise biomimetic total synthesis of incarvilleatone and incarviditone is achieved in one pot via the highly stereoselective hetero- and homodimerization of (±)-rengyolone, respectively. The structure of incarviditone is revised on the basis of spectroscopic and computational evidence.

Dimeric natural products have been recognized as a group of attractive targets for synthetic chemists, because of their fascinating molecular architectures and prominent biological profiles.[1] Not surprisingly, significant efforts have been devoted to their synthesis. Among various approaches to access the dimeric targets, bioinspired strategies which usually exploit a biomimetic dimerization reaction as the key step are of particular interest, since they take advantage of the symmetrical elements of the targets and, thus, dramatically simplify their synthesis.[2]

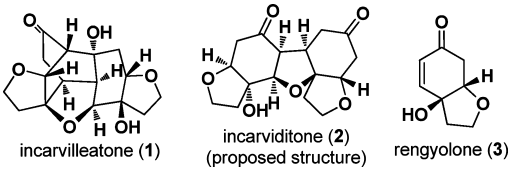


Figure 1. Structure of incarvilleatone (**1**) and incarviditone (**2**).

Incarvilleatone (**1**, Figure 1), a cyclohexylethanoid dimer, was recently isolated by Zhang et al. from *incarvillea younghuibandii*, a plant used as a Chinese folk medicine to treat dizziness and anemia.[3] Preliminary biological

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(1) For leading reviews on the topic of dimeric natural products, see: (a) Lian, G. Y.; Yu, B. *Chem. Biodiversity* **2010**, *7*, 2660–2691. (b) Vrettou, M.; Gray, A. A.; Brewer, A. R. E.; Barrett, A. G. M. *Tetrahedron* **2007**, *63*, 1487–1536. (c) Voloshchuk, T.; Farina, N. S.; Wauchope, O. R.; Kiprowska, M.; Haberfield, P.; Greer, A. *J. Nat. Prod.* **2004**, *67*, 1141–1146.

(2) For selected examples, see: (a) Li, C.; Lobkovsky, E.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 10484–10485. (b) Lei, X.; Johnson, R. P.; Porco, J. A., Jr. *Angew. Chem., Int. Ed.* **2003**, *42*, 3913–3917. (c) Myers, A. G.; Herzon, S. B. *J. Am. Chem. Soc.* **2003**, *125*, 12080–12081. (d) Zhang, W.; Luo, S.; Chen, Q.; Hu, H.; Jia, X.; Zhai, H. *J. Am. Chem. Soc.* **2005**, *127*, 18–19. (e) Nicolaou, K. C.; Lim, Y. H.; Papageorgiou, C. D.; Piper, J. L. *Angew. Chem., Int. Ed.* **2005**, *44*, 7917–7921. (f) Baran, P. S.; Hafensteiner, B. D.; Ambhaikar, N. B.; Guerrero, C. A.; Gallagher, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 8678–8693. (g) Movassaghi, M.; Schmidt, M. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 3725–3728. (h) Li, Z. T.; Gao, Y. X.; Tang, Y. F.; Dai, M. J.; Wang, G. X.; Wang, Z. G.; Yang, Z. *Org. Lett.* **2008**, *10*, 3017–3020. (i) Snyder, S. A.; Kontes, F. *J. Am. Chem. Soc.* **2009**, *131*, 1745–1752. (j) Li, C.; Dian, L. Y.; Zhang, W. D.; Lei, X. G. *J. Am. Chem. Soc.* **2012**, *134*, 12414–12417.

(3) Gao, Y. P.; Shen, Y. H.; Zhang, S. D.; Tian, J. M.; Zeng, H. W.; Ye, J.; Li, H. L.; Shan, L.; Zhang, W. D. *Org. Lett.* **2012**, *14*, 1954–1957.

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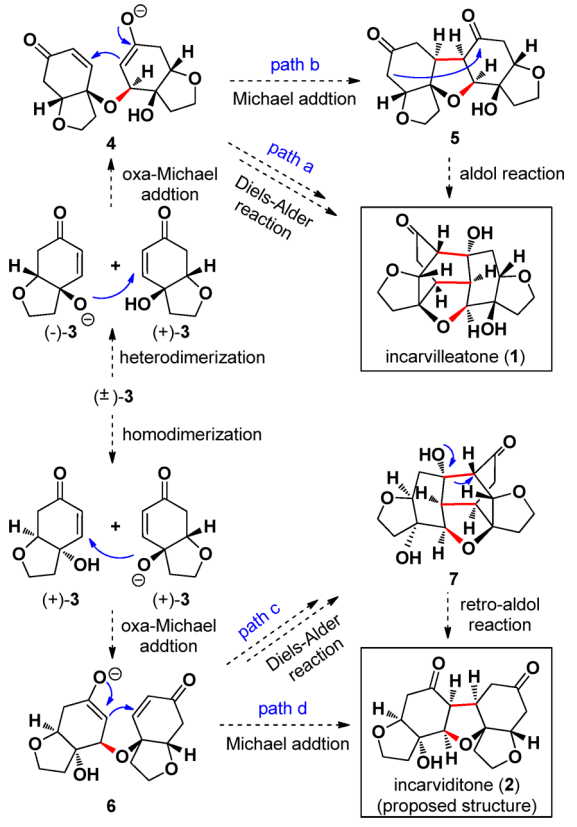
evaluation showed that incarvilleatone displayed a potent inhibitory effect against NO production in LPS-induced RAW264.7 macrophages, rendering it an attractive target for further biomedical studies. Structurally, incarvilleatone (**1**) features a highly congested, cage-shaped hexacyclic skeleton, which poses a synthetic challenge to chemists. Interestingly, incarviditone (**2**), another dimeric natural product biogenetically relevant to incarvilleatone (**1**), was also discovered by the same group from *incarvillea delavayi*.[4] Biogenetically, both **1** and **2** are assumed to be derived from (±)-rengyolone (**3**), a coexisting substance found in the same species as **1** and **2**.[3,4]

As suggested by Zhang et al., incarvilleatone (**1**) might be generated from the heterodimerization of (±)-**3** through a sequential oxa-Michael addition and Diels–Alder reaction (path a, Scheme 1).[3] Alternatively, we envisioned that

Scheme 1. Proposed Biosynthetic Pathway of Incarvilleatone (**1**) and Incarviditone (**2**)



a cascade oxa-Michael/Michael/aldol reaction sequence (path b, Scheme 1) could also account for the transformations from (±)-**3** to **1**.[5] Meanwhile, we postulated that a

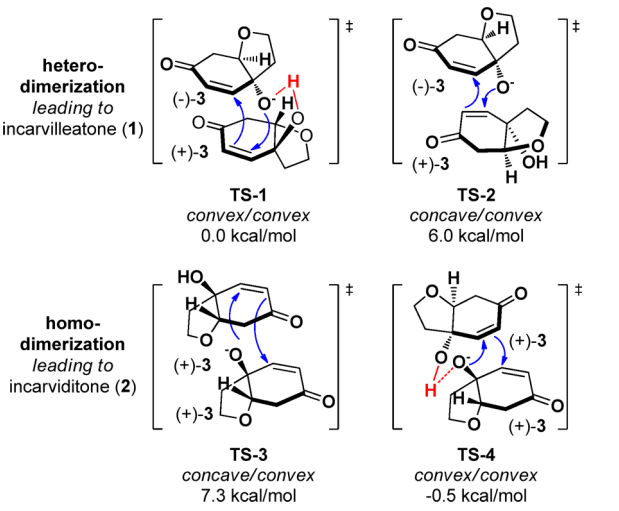


Figure 2. Transition structures (with relative free energy) of the stereodetermining step.

homodimerization of (±)-**3** (e.g., (+)-**3** + (+)-**3**) would afford incarviditone (**2**) through either a cascade oxa-Michael/Diels–Alder/retro-aldol reaction sequence (path c, Scheme 1) or a tandem oxa-Michael/Michael addition reaction (path d, Scheme 1).

As part of our continued interests in the total synthesis of bioactive natural products with unique molecular architectures and intriguing biogenetic pathways, we launched a program toward the synthesis of **1** and **2**. Strategically, we envisioned that the most elegant and efficient approach to make these targets would rely on a biomimetic strategy, through which both **1** and **2** could be generated from (±)-**3** in one pot via hetero- and homodimerization, respectively.

However, further analysis indicated that the biomimetic strategy may be challenging, or even impossible. One of the major concerns stems from the exceptional stereoselectivity involved in the initial step of the putative biosynthetic pathways. As shown in Figure 2, there are four possible transition structures (**TS-1** to **TS-4**) for the oxa-Michael addition, which could probably generate at most four stereoisomers. To gain deeper insight into the stereochemical outcomes of the dimerization processes, computational studies were performed.[7] B3LYP/6-31+G* calculations revealed that, for the heterodimerization, **TS-1**, which leads to the formation of incarvilleatone (**1**), is 6.0 kcal/mol lower in energy than **TS-2**. For the case of homodimerization, **TS-3**, which leads to the generation of incarviditone (**2**), is 7.8 kcal/mol less favorable than **TS-4**. Both convex/convex facial arrangement and hydrogen bonding were found to contribute to the energy preferences of **TS-1** over **TS-2** and **TS-4** over **TS-3**. The computational findings rationalize the stereochemical outcome of the heterodimerization of (±)-**3** but cast doubt on that of the homodimerization process. Even though **TS-3** cannot be

(4) Chen, Y. Q.; Shen, Y. H.; Su, Y. Q.; Kong, L. Y.; Zhang, W. D. *Chem. Biodiversity*. **2009**, *6*, 779–783.

(5) Although the proposed intermediate **5** has to date not been reported as a natural product, computational studies suggested the stepwise mechanism via a tandem Michael/aldol reaction is more likely involved in the transformation from **4** to **1** than the concerted one via a Diels–Alder reaction (for details, see SI).

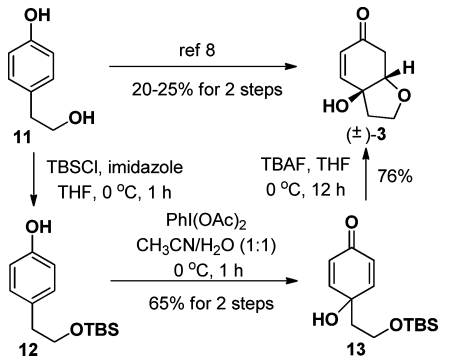
(6) Calculations were performed on B3LYP/6-31+G(d) with Gaussian 09. For details of discussion, see SI.

(7) Carreño, M. C.; González-López, M.; Urbano, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 2737–2741.

excluded in an enzyme catalyzed reaction, it would be hard to envision its involvement in a practice of synthesis. Instead, we hypothesized that incarviditone (**2**) might be generated from the homodimerization of (±)-**3** via **TS-4** instead of **TS-3**. If this hypothesis is correct, the structure of incarviditone (**2**) may be incorrectly assigned in the original isolation paper[4] and require revision.

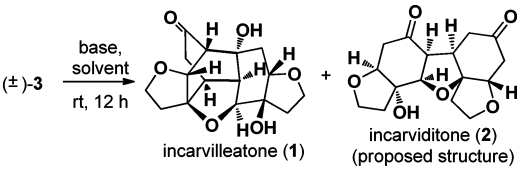
Keeping this hypothesis in mind, we pursue the biomimetic total synthesis of **1** and **2**. The monomer (±)-**3** was initially prepared from commercially available **11** by employing the known method through a sequence of oxidative dearomatization, reduction of the peroxy functionality, and intramolecular oxa-Michael addition (Scheme 2).[7] However, scale-up of this sequence proved to be problematic, and the overall yield of (±)-**3** was only 20−25%. Therefore, a more practical and scalable procedure was developed, which involved three synthetic operations: (1) selective TBS-protection of the primary alcohol of **11** to afford **12**, (2) dearomatization of **12** utilizing $PhI(OAc)_2$ to provide **13**,[8] and (3) deprotection of **13** with TBAF followed by intramolecular conjugate addition to yield (±)-**3**. This procedure enabled the multigram-scale synthesis of (±)-**3** in 50% overall yield for three steps.

Scheme 2. Synthesis of the Monomer (±)-Rengyolone (**3**)



With (±)-rengyolone (**3**) in hand, we started to explore conditions that could promote the proposed dimerization reaction. Commonly used base/solvent combinations[9] which have been applied to promote the intermolecular oxa-Michael addition,[10] such as DABCO/dioxane/H_2O, DBU/DCM, *t*-BuOK/THF, NaHMDS/THF, and NaH/THF, were examined, but none of them provided satisfying results (Table 1, entries 1−5) (for more details of condition screening, see Supporting Information (SI)). Inspired by

Table 1. Condition Screening of the Dimerization of (±)-Rengyolone (**3**)



entry	base	solvent	yield of **1** (%)	yield of **2** (%)
1	DABCO (excess)	dioxane/H_2O	NR	NR
2	DBU (excess)	DCM	NR	NR
3	*t*-BuOK (2.0 equiv)	THF	15[a]	17[a]
4	NaHMDS (2.0 equiv)	THF	0[a]	7[a]
5	NaH (2.0 equiv)	THF	0[a]	0[a]
6	NaH (2.0 equiv)	DCM	25[b]	30[b]
7	NaH (1.0 equiv)	DCM	38[b]	40[b]
8	NaH (0.5 equiv)	DCM	30[b]	35[b]

[a] Refers to ^{1}H NMR yield with 1,3,5-trimethylbenzene as the internal standard. [b] Refers to isolated yield. DABCO = 1,4-diazabicyclo[2.2.2]-octane. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene. NaHMDS = sodium bis(trimethylsilyl)amide.

the work of Carreño,[11] in which the homodimerization and trimerization of a *p*-quinol type of substrates via successive conjugate additions were well investigated, we tried the NaH/DCM combination in this reaction. To our delight, treatment of (±)-**3** with NaH (2.0 equiv) in DCM for 12 h afforded two major products in yields of 25% and 30%, respectively (entry 6). One of them was shown to be carvilleatone (**1**) based on the good agreement of its spectroscopic data (^{1}H NMR, ^{13}C NMR, and HRMS) with those of the natural product[3] as well as the X-ray crystallographic study.[12] The other, as will be discussed in more detail below, was incarviditone.

Although only modest yields were obtained, the results demonstrated the feasibility of the biomimetic strategy. Encouraged by these results, we examined the effect of other reaction parameters on the transformation. Although substantial amounts of starting material were recovered in the reaction listed in entry 6, efforts to improve the conversion by extending the reaction time, elevating the reaction temperature, or using a large excess of NaH only led to the formation of some unidentified products. In sharp contrast, when the dimerization reaction was performed with a reduced amount of NaH (1.0 equiv), **1** and **2** were obtained in a satisfactory yield of 38% and 40%, respectively, accompanied with the recovery of 10% of the starting material (entry 7). Furthermore, we found that even substoichiometric amounts (0.5 equiv) of NaH could effectively promote the transformation, with only a lightly decreased yield of **1** and **2** obtained (entry 8).[13] No stereoisomers of **1**

(8) You, Z.; Hoveyda, A. H.; Snapper, M. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 547–550.

(9) For selected examples of base-promoted intermolecular oxa-Michael addition, see: (a) Dumez, E.; Rodriguez, J.; Dulcère, J.-P. *Chem. Commun.* **1997**, 1831–1832. (b) Enders, D.; Haertwig, A.; Raabe, G.; Runsink, J. *Eur. J. Org. Chem.* **1998**, 1771–1792. (c) Lesch, B.; Bräse, S. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 115–118. (d) Nising, C. F.; Ohnemüller, U. K.; Bräse, S. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 307–309. (e) Xiong, X.; Ovens, C.; Pilling, A. W.; Ward, J. W.; Dixon, D. J. *Org. Lett.* **2008**, *10*, 565–567.

(10) For reviews on the topic of oxa-Michael addition, see: (a) Nising, C. F.; Bräse, S. *Chem. Soc. Rev.* **2008**, *37*, 1218–1228. (b) Nising, C. F.; Bräse, S. *Chem. Soc. Rev.* **2012**, *41*, 988–999.

(11) Carreño, M. C.; Ribagorda, M. *Org. Lett.* **2003**, *5*, 2425–2428.

(12) For X-ray crystallagraphic data of **1**, see SI.

(13) For a detailed discussion on the dimerization of (±)-(**3**) with substoichiometric amounts of NaH, see SI.

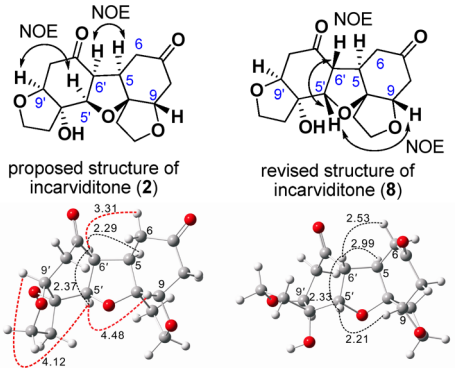


Figure 3. Key NOE correlations and DFT optimized geometries of proposed structure (**2**) and revised structure (**8**) (distances are in Å).

or **2** or the intermediates **5** and **7** (Scheme 1) were identified under the optimal conditions, indicating that both the hetero- and homodimerization of (±)-(**3**) proceeded through a cascade reaction with exceptionally high stereoselectivity.[14]

With enough synthetic material in hand, we began to test our hypothesis about the structure assignment of incarviditone. We noticed that in the original isolation paper the relative stereochemistries of C-5′ and C-6′ of **2** were assigned as an α-configuration, mainly because of the observed cross-peaks of H−C(6′) with H−C(5) and H−C(5′) with H−C(9′) in the NOESY correlation. However, such assignments might be misleading due to two facts: (1) H−C(6′) and H−C(5) are located at adjacent carbons of a five-membered ring, and thus the observed NOESY signal between them cannot unequivocally prove their cis-relationship; (2) the ^{1}H NMR signal of H−C(9′) largely overlaps with that of H−C(9) in the documented NOESY spectrum (in $CDCl_3$), and thus the observed cross-peak of H−C(5′) with H−C(9′) could be instead attributed to that of H−C(5′) with H−C(9).[15] In fact, both H--H distances of H−C(5′)/H−C(9′) and H−C(5′)/H−C(9) (4.12 Å or 4.48 Å) in the B3LYP/6-31+G(d) optimized geometry of **2** are too long to show the NOESY correlation (Figure 3). Additional evidence came from the following NMR experiments: when the NOESY spectrum of synthetic incarviditone was collected in CD_3OD, the signals of H−C(9) and H−C(9′) could be well differentiated from each other and the observed NOESY cross-peaks of H−C(5′) with H−C(9), in conjunction with the corresponding short H--H distances (2.21 Å) in the DFT optimized structures (Figure 3), clearly suggested that H−C(5′) and H−C(6′) should be oriented in a β-configuration. Fortunately, as more direct and conclusive evidence, the X-ray crystal structure of **14** (for its preparation, see SI), a derivative of incarviditone, was obtained, which unambiguously confirmed the above stereochemical assignments (Figure 4).[16] As such, we concluded that the structure of incarviditone should be corrected to **8** (Figure 3).

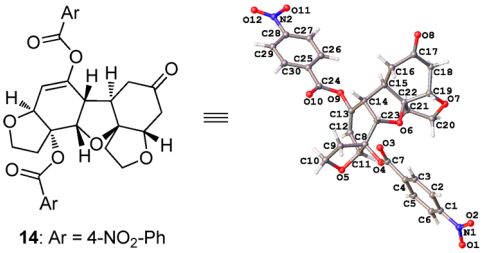


Figure 4. X-ray crystal structure of **14**.

In summary, we have achieved a concise, biomimetic, and one-pot total synthesis of incarvilleatone (**1**) and incarviditone (**8**) via the highly stereoselective hetero- and homodimerization of (±)-rengyolone (**3**), respectively. Furthermore, the structure of incarviditone is revised based on computational studies and spectroscopic evidence. This work showcases how a proposed biogenetic pathway could be implemented in the laboratory environment by judicious choice of reaction conditions and, in turn, how the practice of biomimetic synthesis can help elucidate the underlying biosynthetic pathways of the targets.

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Supporting Information Available. Computational details, experimental procedure, and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(14) Following the reviewer's suggestion, the purified incarvilleatone and incarviditone were respectively treated with 1.0 equiv of NaH in DCM to see whether there exists an equilibrium between them. It turned out that no new spot could be observed on the TLC, which suggested such an equilibrium could be precluded under the current conditions.

(15) Careful analysis of the authentic NMR spectrum of incarviditone, which was kindly provided by Prof. Zhang, revealed that the signals of H-C(9) and H-C(9′) were misassigned to each other in the original isolation paper (for details, see SI).

(16) CCDC 897020 contains the supplementary crystallographic data for **14**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www. ccdc.cam.ac.uk/data_request/cif.

The authors declare no competing financial interest.