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Total synthesis of incargranine A†

Patrick D. Brown and Andrew L. Lawrence 🕩 *

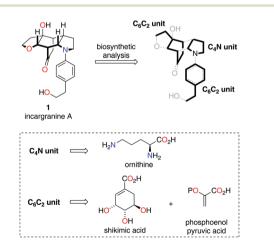
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Synthetic studies into the origins of the alkaloid incargranine A have resulted in the development of a four-step (longest linear sequence) total synthesis. This synthesis has been scaled-up to provide gram-scale quantities of material, which would alternatively require extraction of several metric-tons of dried-whole Chinese Trumpet-Creeper plants (*Incarvillea mairei* var. *grandiflora*).

In 2009 Zhang and co-workers isolated the alkaloid incargranine A (1) from *Incarvillea mairei* var. *grandiflora*, a Bignonia plant more commonly known as the Chinese Trumpet-Creeper plant (Scheme 1).¹ Incargranine A (1) has not yet succumbed to total synthesis and represents a particularly scarce natural product, constituting just 0.0000002% by weight of the dried whole plant. Therefore, a practical – *i.e.*, efficient and scalable – chemical synthesis of incargranine A (1) might advance a



Scheme 1 Structure and biosynthetic analysis of incargranine A.

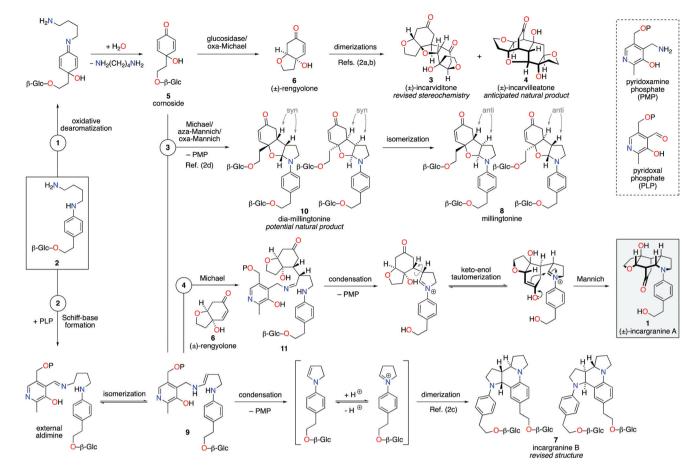
better understanding of its biological function. The novel framework of incargranine A (1) contains a synthetically daunting bridged-cyclohexane ring, in which all six-carbon atoms are stereogenic. Nevertheless, we were hopeful that if we could gain insight into how nature synthesizes this alkaloid a stepeconomical biomimetic strategy could be developed.

Our biosynthetic analysis, shown in Scheme 1, reveals incargranine A (1) is likely constructed from two shikimatederived C₆C₂ units linked together by an ornithine-derived C₄N unit. Our previous biomimetic studies on related phenylethanoid alkaloids provide important clues as to the potential origins of incargranine A (1)² We recently proposed that a network of pathways, all originating from a simple biosynthetic precursor, diamine 2, could account for the formation of several structurally distinct phenylethanoid natural products (Scheme 2).^{2d} In our proposal, diamine 2 can participate in a pair of divergent oxidative pathways (Scheme 2; pathways 1 and 2). As shown in Scheme 2, pathway 1 terminates in the formation of incarviditone $(3)^3$ and incarvilleatone $(4)^4$ via the intermediacy of cornoside $(5)^5$ and rengyolone (6),⁶ whereas pathway 2 results in the production of incargranine B (7).^{2*a*-*c*,7} It was proposed that these two divergent pathways could reconverge to give millingtonine (8),8 via a crossed-dimerization of cornoside 5, from pathway 1, and a PLP (pyridoxal phosphate) derived enamine 9, from pathway 2 (Scheme 2; pathway 3).^{2d} The chemical feasibility of this re-convergent pathway was demonstrated in our seven-step biomimetic total synthesis of millingtonine (8).^{2d} Herein, we propose that an additional reconvergent pathway could give rise to incargranine A (1) (Scheme 2; pathway 4). Thus, a Michael reaction between PLPenamine 9 and rengyolone (6) would give an intermediate imine 11, which would ring-close through a condensation/ Mannich reaction sequence to give incargranine A (1).9 To investigate the feasibility of this second re-convergent pathway, and in the hope of establishing a practical solution to the supply problem associated with incargranine A (1),¹ we decided to pursue the development of a biomimetic synthetic strategy.

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EaStCHEM School of Chemistry, University of Edinburgh, Joseph Black Building, David Brewster Road, Edinburgh, EH9 3FJ, UK. E-mail: a.lawrence@ed.ac.uk †Electronic supplementary information (ESI) available. See DOI: 10.1039/ c8ob00702k

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Scheme 2 Proposed network of biosynthetic pathways towards a family of plant-derived phenylethanoid natural products, including incargranine A.

Condensation of 4-aminophenethyl alcohol 12 with (Z)-1,4dichlorobut-2-ene gave N-aryl-2,5-dihydropyrrole 13 in 87% yield (Scheme 3).¹⁰ The primary alcohol functional group was then protected under standard conditions as a tert-butyldimethylsilyl ether, to give alkene 14 in 84% yield. Exposure of alkene 14 to our previously developed RhHCO(PPh₃)₃ and pyrrolidine reaction conditions gave the expected aminal intermediate 15.^{2d,11} Due to the instability of aminal 15, and in the interests of practicality and efficiency, rengyolone (6), which can be readily prepared from tyrosol in 3 steps,^{2a} was added directly to this crude reaction mixture. Monitoring the reaction by ¹H NMR spectroscopy revealed it took 10 days at ambient temperature for aminal 15 to be consumed. Purification of the resulting crude reaction mixture by column chromatography resulted in a 12% isolated yield of an unwanted crossed-dimer 16, with no detectable formation of the desired product 17. Hemi-aminal 16 is presumably formed via a domino Michael/aza-Mannich/oxa-Mannich reaction sequence. In contrast, a final Mannich reaction between C5 and C1" would be required for formation of the incargranine A framework 17 (Scheme 3). Although this result demonstrates the viability of a crossed-dimerization between aminal 15 and rengyolone (6), several issues presented themselves with respect to using this strategy to access incargranine A (1). Firstly, rengyolone (6) proved to be relatively unreactive in the crossed-dimerization, taking over a week to give full consumption of starting material 15, while comparable reactions with *para*-quinols were generally complete in 24 h.^{2d} Furthermore, the low yield of crossed-dimer 16, even after these prolonged reaction times, was not a promising start to the development of an efficient synthesis. Finally, and most importantly, our attempts to rearrange hemi-aminal 16 to give the incargranine A framework 17, *via* a retro-oxa-Mannich/Mannich reaction sequence, were unsuccessful.¹² This prompted us to reconsider our biosynthetic proposal and synthetic strategy.

Upon further evaluation of the incargranine A (1) framework it became apparent that it might instead be derived from the *syn*-diastereomer of millingtonine, dia-millingtonine (10), which we had previously identified as a potential natural product and direct biosynthetic precursor to millingtonine (8) (Scheme 2; pathway 3).^{2d} Specifically, the putative aglycone of dia-millingtonine, diol 18, could undergo a domino retro-oxa-Mannich/oxa-Michael/Mannich reaction sequence to give incargranine A (1) (Scheme 4).¹³ If this pathway could be shown to be chemically feasible it would lend further support

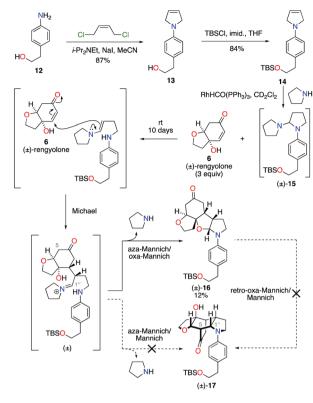
(±)-**21**

[2.8 g scale]

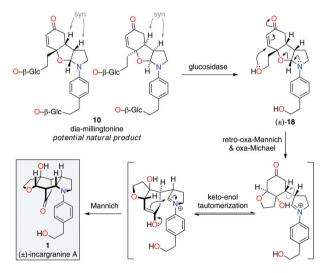
I₂, MeOH

84% [1.3 g scale]

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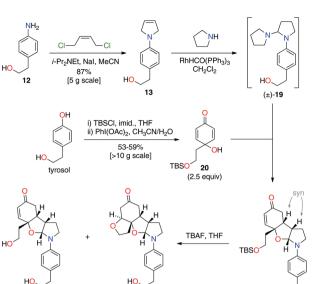
Scheme 3 Failed approach to synthesize incargranine A.



Scheme 4 Revised biosynthetic hypothesis for incargranine A.

to our proposal that dia-millingtonine (1) represents an as-yetundiscovered natural product.^{2d}

During the development of this new strategy, it was discovered that protection of the primary alcohol in *N*-aryl-2,5-dihydropyrrole **13** was not necessary for the subsequent alkeneisomerization/hydroamination reaction. Thus, exposure of free alcohol **13** to RhHCO(PPh₃)₃ and pyrrolidine gave the expected aminal intermediate **19** (Scheme 5).^{2d,11} TBS-protected *para*-



(+)-22

Scheme 5 Total synthesis of incargranine A.

CD₃OD, 40 °C, 55 h

33%

(±)-18

10%

quinol **20**, which was prepared in 2 steps from tyrosol,^{2*a*} was then added directly to this crude reaction mixture resulting in a kinetically-controlled crossed-dimerization to give *syn*-dimer **21** in 77% yield.^{2*d*}

(±)-incargranine A

Attention could now turn to the de-protection of crosseddimer 21, a synthetic equivalent of dia-millingtonine (10), and its subsequent conversion to incargranine A (1). Cleavage of the tert-butyldimethylsilyl ether using standard TBAF (tetra-nbutylammonium fluoride) conditions gave the expected diolaglycone 18 in just 10% yield, alongside a cyclized-aglycone 22 in 56% yield (Scheme 5). Remarkably, it was observed that diol-aglycone 18 spontaneously rearranges to give (\pm) -incargranine A (1) when dissolved in methanol at ambient temperature, albeit very slowly. Ultimately, a 33% isolated yield of (±)-incargranine A (1) was achieved when a CD_3OD solution of diol-aglycone 18 was warmed to 40 °C for 2 days. The chemical feasibility of our proposed biosynthetic pathway between diamillingtonine (10) and incargranine A (1) had thus been established. All efforts, however, to rearrange the cyclized-aglycone 22 to give incargranine A (1) were unsuccessful, akin to our failure to rearrange hemi-aminal 16 (Scheme 3).¹²

The low yields and lack of selectivity achieved in the final de-protection and rearrangement steps rendered this synthesis unsuitable for scale-up. Alternative deprotection conditions were therefore screened in the hope of favoring production of diol **18**, whilst avoiding formation of the seemingly intractable ring-closed aglycone **22**. Vaino and Szarek have reported iodine in methanol as mild reaction conditions for the cleavage of *tert*-butyldimethylsilyl ethers.¹⁴ Unexpectedly, however, exposure of *syn*-dimer **21** to iodine in methanol did not result in the formation of diol **18**, nor ring-closed aglycone **22**, but instead gave (\pm)-incargranine A (**1**) directly. Thus, in a single step, 2 new bonds, 2 new rings and 3 new stereogenic centres are formed in an impressive 84% yield. This synthetic sequence was readily scaled-up to provide gram-scale quantities of (\pm)-incargranine A (**1**), which compares very favorably to the effort required to obtain this material from the natural source; over four metric-tons of dried *Incarvillea mairei* var. *grandiflora* would need to be extracted to isolate one gram of natural incargranine A (**1**).¹

Zhang and co-workers reported an optical rotation for natural incargranine A (1), $[\alpha]_{D}^{22} = +2$ (c = 0.175, CHCl₃).¹ However, given our biosynthetic speculation and the small magnitude of the reported optical rotation value, we consider it likely that natural incargranine A (1) exists as a racemic mixture. Unfortunately, no authentic sample was available to validate this hypothesis.¹⁵ In all other respects, however, the spectroscopic data for our synthetic material matched that reported for natural incargranine A (1).^{1,15} We propose that this successful synthesis provides new evidence in support of the proposal that dia-millingtonine (10) is a natural product.^{2d,16} In fact, it is possible that incargranine A (1) is only produced from dia-millingtonine (10) during the extraction and isolation process. This would not necessarily mean that incargranine A (1) is an unimportant artifact of human intervention.¹⁷ It is known, for example, that plants can use glycosidic-metabolites as chemical defense systems, wherein damage to the plant brings gycosidase enzymes into contact with the glycosides to release the active aglycones.¹⁸

Conclusions

In just three-linear steps from 4-aminophenethyl alcohol **12** we have selectively formed 2 new C–N bonds, 2 new C–C bonds, 2 new rings, and 6 new contiguous stereogenic centres, in 56% overall yield.¹⁹ Key to the development of this efficient synthetic strategy has been the probing and refinement of a bio-synthetic proposal using chemical synthesis. Ultimately, this has led to new evidence in support of the notion that diamillingtonine (**10**) is an as-yet-undiscovered natural product.¹⁶ Practical quantities of these metabolites are now available for interested parties to study their biological function.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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of further rearrangement. Treatment with LiOH in refluxing $MeOH/H_2O$ resulted in slow decomposition.

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