Total synthesis of incargranine A†

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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). 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 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 step-economical biomimetic strategy could be developed.

Our biosynthetic analysis, shown in Scheme 1, reveals incargranine A (1) is likely constructed from two shikimate-derived 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). 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). 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) and incarvilleatone (4), via the intermediacy of cornoside (5) and rengyolone (6), whereas pathway 2 results in the production of incargranine B (7). It was proposed that these two divergent pathways could reconverge to give millingtonine (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). The chemical feasibility of this re-convergent pathway was demonstrated in our seven-step biomimetic total synthesis of millingtonine (8). Herein, we propose that an additional re-convergent pathway could give rise to incargranine A (1) (Scheme 2; pathway 4). Thus, a Michael reaction between PLP-enamine 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). 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), we decided to pursue the development of a biomimetic synthetic strategy.

Condensation of 4-aminophenethyl alcohol 12 with (Z)-1,4-dichlorobut-2-ene gave N-aryl-2,5-dihydropyrrrole 13 in 87%...
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. Due to the instability of aminal 15, a di-nthe interests of practicality and efficiency, rengyolone (6), which can be readily prepared from tyrosol in 3 steps, 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. 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. 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). 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). If this pathway could be shown to be chemically feasible it would lend further support to our proposal that dia-millingtonine (1) represents an as-yet-undiscovered natural product.
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 alkene-isomerization/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-quinol 20, which was prepared in 2 steps from tyrosol,2a was then added directly to this crude reaction mixture resulting in a kinetically-controlled crossed-dimerization to give syn-dimer 21 in 77% yield.2d

Attention could now turn to the de-protection of crossed-dimer 21, a synthetic equivalent of dia-millingtonone (10), and its subsequent conversion to incarganine A (1). Cleavage of the tert-butylidemethylsilyl ether using standard TBAF (tetra-n-butylammonium fluoride) conditions gave the expected diol-aglycone 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 (+)-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 CD3OD solution of diol-aglycone 18 was warmed to 40 °C for 2 days. The chemical feasibility of our proposed biosynthetic pathway between dia-millingtonone (10) and incarganine A (1) had thus been established. All efforts, however, to rearrange the cyclized-aglycone 22 to give incarganine 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-butylidimethylsilyl ethers.\textsuperscript{14} Unexpectedly, however, exposure of syn-dimer \textsuperscript{21} to iodine in methanol did not result in the formation of diol \textsuperscript{18}, nor ring-closed aglycone \textsuperscript{22}, but instead gave (±)-incargranine \textsuperscript{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 (±)-incargranine \textsuperscript{A} (1), which compares very favorably to the effort required to obtain this material from the natural source; over four metric-tons of dried \textit{Incarvillea mairei} var. \textit{grandiflora} would need to be extracted to isolate one gram of natural incargranine \textsuperscript{A} (1).\textsuperscript{3}

Zhang and co-workers reported an optical rotation for natural incargranine \textsuperscript{A} (1), \([\alpha])_{D}^{22} = +2 (c = 0.175, CHCl\textsubscript{3}).\textsuperscript{1}\) However, given our biosynthetic speculation and the small magnitude of the reported optical rotation value, we consider it likely that natural incargranine \textsuperscript{A} (1) exists as a racemic mixture. Unfortunately, no authentic sample was available to validate this hypothesis.\textsuperscript{15} In all other respects, however, the spectroscopic data for our synthetic material matched that reported for natural incargranine \textsuperscript{A} (1).\textsuperscript{1,15} We propose that this successful synthesis provides new evidence in support of the proposal that dia-millingtonine (\textsuperscript{10}) is a natural product.\textsuperscript{2,16} In fact, it is possible that incargranine \textsuperscript{A} (1) is only produced from dia-millingtonine (\textsuperscript{10}) during the extraction and isolation process. This would not necessarily mean that incargranine \textsuperscript{A} (1) is an unimportant artifact of human intervention.\textsuperscript{17} It is known, for example, that plants can use glycosidic-metabolites as chemical defense systems, wherein damage to the plant brings glycosidase enzymes into contact with the glycosides to release the active aglycones.\textsuperscript{18}

\section*{Conclusions}

In just three-linear steps from 4-aminophenethyl alcohol \textsuperscript{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.\textsuperscript{19} Key to the development of this efficient synthetic strategy has been the probing and refinement of a biosynthetic proposal using chemical synthesis. Ultimately, this has led to new evidence in support of the notion that dia-millingtonine (\textsuperscript{10}) is an as-yet-undiscovered natural product.\textsuperscript{16} Practical quantities of these metabolites are now available for interested parties to study their biological function.

\section*{Conflicts of interest}

There are no conflicts to declare.

\section*{Acknowledgements}

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\section*{Notes and references}


9 For our earliest biosynthetic proposals, see page 36 of the \textit{ESI} for ref. \textit{2c}.


12 Attempts to rearrange hemi-aminals \textit{16} and \textit{22} failed. Heating solutions in MeOH, EtOH or MeCN returned starting material. Treatment with TFA appeared to give isomerization from the \textit{syn} to the \textit{anti} configuration, with no sign of further rearrangement. Treatment with LiOH in refluxing MeOH/H\textsubscript{2}O resulted in slow decomposition.

13 Treatment of millingtonone with glucosidase enzymes has been shown to result in a retro-oxa-Mannich/oxa-Michael/
Mannich reaction sequence to give a diastereomer of incargranine A, see ref. 8.


15 Professor Zhang very kindly provided pdf files of the processed NMR spectra for natural incargranine A, see the ESI† for direct comparisons.


