



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).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**).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**).^{2a-c,7} It was proposed that these two divergent pathways could re-converge 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 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**).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.

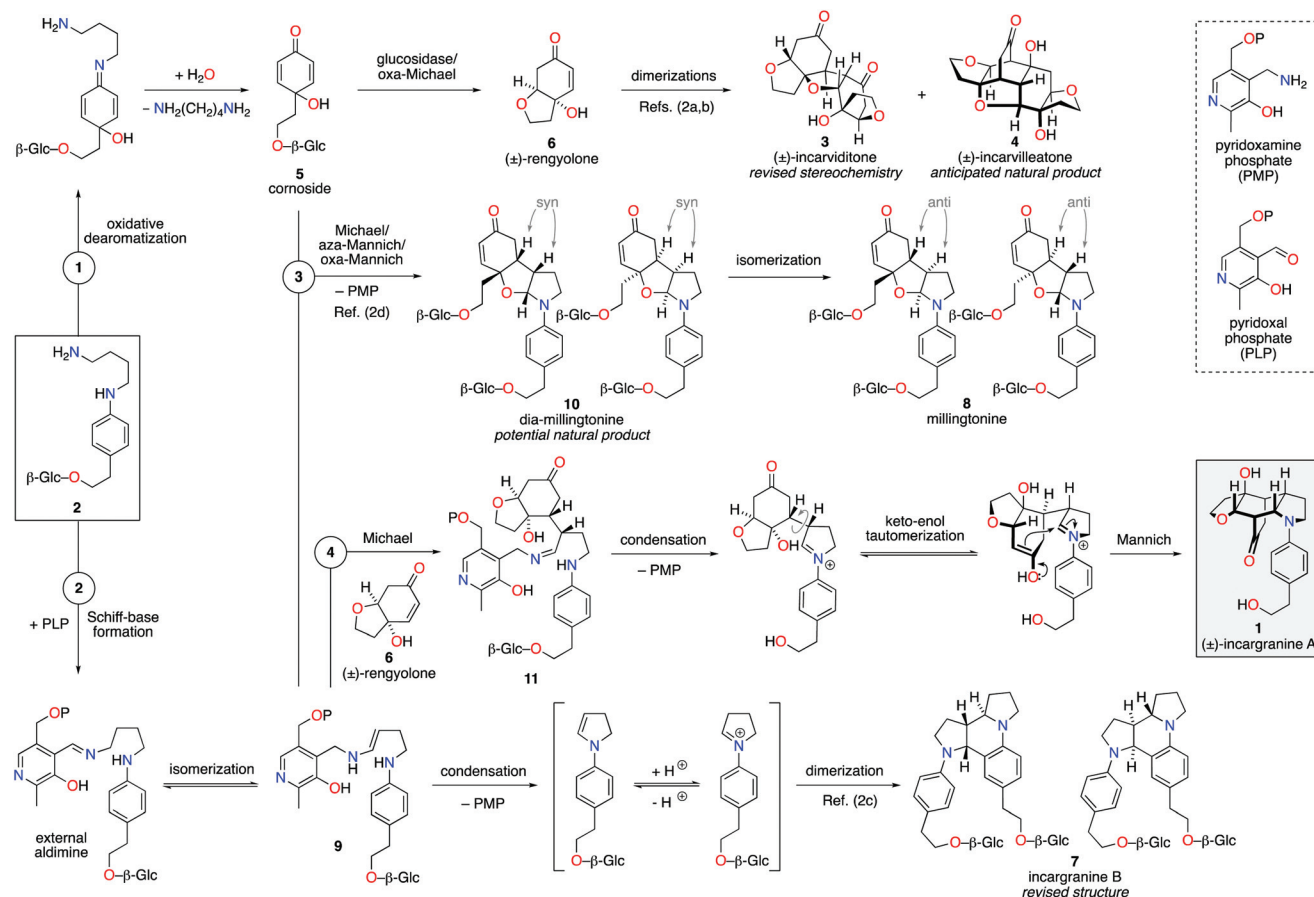


Scheme 1 Structure and biosynthetic analysis of incargranine A.

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

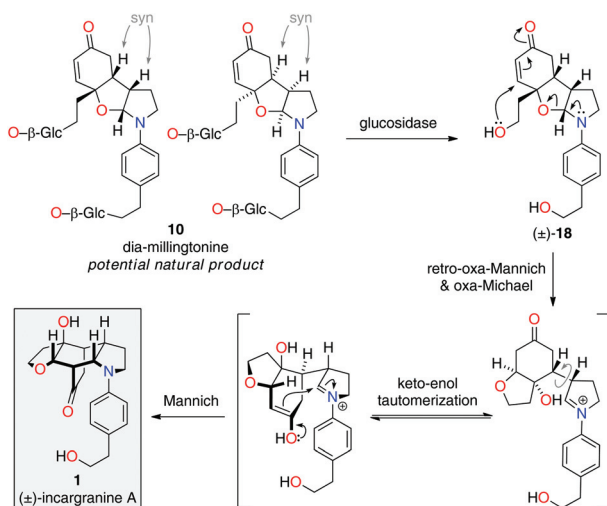
Condensation of 4-aminophenethyl alcohol **12** with (Z)-1,4-dichlorobut-2-ene gave *N*-aryl-2,5-dihydropyrrole **13** in 87% yield (Scheme 3).¹⁰ The primary alcohol functional group was then protected under standard conditions as a *tert*-butyldimethylsilyl ether, to give alkene **14** in 84% yield. Exposure of alkene **14** to our previously developed RhHCO(PPh₃)₃ and pyrrolidine reaction conditions gave the expected amination intermediate **15**.^{2d,11} Due to the instability of amination **15**, and in the interests of practicality and efficiency, renyolone (**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 amination **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-amination **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 amination **15** and renyolone (**6**), several issues presented them-

selves 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



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-yet-undiscovered 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 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*-



Scheme 5 Total synthesis of incargranine A.

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-millingtonine (10), and its subsequent conversion to incargranine A (1). Cleavage of the *tert*-butyldimethylsilyl 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-millingtonine (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**), $[\alpha]_{\text{D}}^{22} = +2$ ($c = 0.175$, CHCl_3).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 glycosidase 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 biosynthetic proposal using chemical synthesis. Ultimately, this has led to new evidence in support of the notion that dia-millingtonine (**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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Notes and references

- 1 Y.-Q. Su, Y.-H. Shen, S. Lin, J. Tang, J.-M. Tian, X.-H. Liu and W.-D. Zhang, *Helv. Chim. Acta*, 2009, **92**, 165–170.
- 2 For previous biomimetic studies on related dimeric natural products, see: (a) P. D. Brown, A. C. Willis, M. S. Sherburn and A. L. Lawrence, *Org. Lett.*, 2012, **14**, 4537–4539; (b) K. Zhao, G.-J. Cheng, H. Yang, H. Shang, X. Zhang, Y.-D. Wu and Y. Tang, *Org. Lett.*, 2012, **14**, 4878–4881; (c) P. D. Brown, A. C. Willis, M. S. Sherburn and A. L. Lawrence, *Angew. Chem.*, 2013, **125**, 13515–13517, (*Angew. Chem. Int. Ed.*, 2013, **52**, 13273–13275); (d) P. D. Brown and A. L. Lawrence, *Angew. Chem.*, 2016, **128**, 8561–8565, (*Angew. Chem. Int. Ed.*, 2016, **55**, 8421–8425).
- 3 Y. Q. Chen, Y. H. Shen, Y. Q. Su, L. Y. Kong and W. D. Zhang, *Chem. Biodiversity*, 2009, **6**, 779–783.
- 4 Y. P. Gao, Y. H. Shen, S. D. Zhang, J. M. Tian, H. W. Zeng, J. Ye, H. L. Li, L. Shan and W. D. Zhang, *Org. Lett.*, 2012, **14**, 1954–1957.
- 5 J. S. Rosendal, A. Kjaer and N. B. Juhl, *Acta Chem. Scand.*, 1973, **27**, 367–369.
- 6 (a) K. Endo and H. Hikino, *Can. J. Chem.*, 1984, **62**, 2011–2014; (b) I. Messana, M. Sperandei, G. Multari, C. Galeffi and G. B. Marini Bettolo, *Phytochemistry*, 1984, **23**, 2617–2619; (c) J. Tian, Q. S. Zhao, H. J. Zhang, Z. W. Lin and H. D. Sun, *J. Nat. Prod.*, 1997, **60**, 766–769.
- 7 Y.-H. Shen, Y.-Q. Su, J.-M. Tian, S. Lin, H.-L. Li, J. Tang and W. D. Zhang, *Helv. Chim. Acta*, 2010, **93**, 2393–2396.
- 8 T. Hase, K. Ohtani, R. Kasai, K. Yamasaki and C. Picheansoonthon, *Phytochemistry*, 1996, **41**, 317–321.
- 9 For our earliest biosynthetic proposals, see page 36 of the ESI for ref. 2c.
- 10 J. M. Bobbitt, L. H. Amundsen and R. I. Steiner, *J. Org. Chem.*, 1960, **25**, 2230–2231.
- 11 (a) W. A. Herrmann and M. Prinz, *Applied Homogeneous Catalysis with Organometallic Compounds*, Wiley-VCH, Weinheim, 2nd edn, 2002; (b) T. J. Donohoe, T. J. C. O'Riordan and C. P. Rosa, *Angew. Chem.*, 2009, **121**, 1032–1035, (*Angew. Chem. Int. Ed.*, 2009, **48**, 1014–1017); (c) A. Vasseur, J. Bruffaerts and I. Marek, *Nat. Chem.*, 2016, **8**, 209–219.
- 12 Attempts to rearrange hemi-aminals **16** and **22** failed. Heating solutions in MeOH, EtOH or MeCN returned starting material. Treatment with TFA appeared to give isomerization from the *syn* to the *anti* configuration, with no sign



- of further rearrangement. Treatment with LiOH in refluxing MeOH/H₂O resulted in slow decomposition.
- 13 Treatment of millingtonine 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.
 - 14 A. R. Vaino and W. A. Szarek, *Chem. Commun.*, 1996, **20**, 2351–2352.
 - 15 Professor Zhang very kindly provided pdf files of the processed NMR spectra for natural incargranine A, see the ESI† for direct comparisons.
 - 16 For recent examples of natural product anticipation from biomimetic studies, see: (a) D. P. O'Malley, K. Li, M. Maue, A. L. Zografos and P. S. Baran, *J. Am. Chem. Soc.*, 2007, **129**, 4762–4775; (b) P. Sharma, B. Lygo, W. Lewis and J. E. Moses, *J. Am. Chem. Soc.*, 2009, **131**, 5966–5972; (c) M. Gavagnin, E. Mollo and G. Cimino, *Rev. Bras. Farmacogn.*, 2015, **25**, 588–591; (d) A. L. Lawrence, R. M. Adlington, J. E. Baldwin, V. Lee, J. A. Kershaw and A. L. Thompson, *Org. Lett.*, 2010, **12**, 1676–1679; (e) V. Sofiyev, J.-P. Lumb, M. Volgraf and D. Trauner, *Chem. – Eur. J.*, 2012, **18**, 4999–5005; (f) S. Strych, G. Journot, R. P. Pemberton, S. C. Wang, D. J. Tantillo and D. Trauner, *Angew. Chem.*, 2015, **127**, 5168–5172, (*Angew. Chem. Int. Ed.*, 2015, **54**, 5079–5083); (g) P. Ellerbrock, N. Armanino, M. K. Ilg, R. Webster and D. Trauner, *Nat. Chem.*, 2015, **7**, 879–882.
 - 17 P. Champy, Artifacts and Natural Substances Formed Spontaneously, in *Biomimetic Organic Synthesis*, ed. E. Poupon and B. Nay, Wiley-VCH, Weinheim, 2011, vol. 2, pp. 849–934.
 - 18 A. Mithofer and W. Boland, *Annu. Rev. Plant Biol.*, 2012, **63**, 431–450.
 - 19 N. J. Green and M. S. Sherburn, *Aust. J. Chem.*, 2013, **66**, 267–283.

