Published in final edited form as:

Nature. 2014 May 15; 509(7500): 318–324. doi:10.1038/nature13273.

Total synthesis and isolation of citrinalin and cyclopiamine congeners

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Abstract

It is said that carbon, the most abundant element in organic matter, supplies life's quantity, whereas nitrogen supplies its quality. It is therefore unsurprising that many natural products that contain basic nitrogens (alkaloids) are coveted for their benefit to human health. However, nitrogen is known to mire many chemical syntheses because of its basicity and susceptibility to oxidation. This challenge may be heightened by the presence of more than one nitrogen atom in a targeted complex alkaloid, but can be met by the selective introduction and removal of functional groups that mitigate basicity, as highlighted herein with the first chemical syntheses of citrinalin B

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Author Contributions R.S. conceived and directed the synthetic aspects of the research, as well as composed the majority of the manuscript (with input from all authors) except the section on biosynthesis, which was contributed by S.R., E.F.P., D.E.W., R.J.A. and R.G.S.B. The synthetic plan was designed by R.S. with input from E.V.M.M. and P.G.R. who also executed the plan under the supervision of R.S. Oxidation catalyst **32** was provided by D.K.R. and S.J.M., who along with P.G.R, E.V.M.M. and R.S. designed the oxidation studies of **25**, which were executed by P.G.R. The computational NMR predictions for **1, 2** and **3** were designed and executed by M.W.L. and D.J.T. with input from P.G.R., E.V.M.M. and R.S. Biosynthetic studies were designed and conducted by S.R., E.F.P. and R.G.S.B. who also isolated and characterized **3**, **37**, and **38**. D.E.W. and R.J.A. provided facilities and contributed to the purification, data analysis and structural analysis of **3**, **37**, and **38**.

Author Information *P. citrinum* F53 is deposited at the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI) under the accession code CBMAI 1186. CCDC 984477, 984478, 984480 and 984479 contains the supplementary crystallographic data for crystal structures *ent*-**2**•HCl, **6**, **27** and **36**, respectively. 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 interests. Readers are welcome to comment on the online version of this article at www.naturecom/nature.

and cyclopiamine B. The chemical connections that have been realized as a result of these syntheses, in addition to the isolation of both 17-hydroxycitrinalin B and citrinalin C through ^{13}C feeding studies, supports the existence of a common bicyclo[2.2.2]diazaoctane containing biogenetic precursor to these compounds as has been proposed previously.

Introduction

The prenylated indole alkaloids are an emerging class of natural products typified by the presence of an indole ring, or derivatives thereof (i.e., spirooxindole or pseudoindoxyl), decorated by one or more prenyl groups or the vestige of a prenyl group. Isolates from this family of natural products include citrinalins A and B (**1** and **2**, see Figure 1) and cyclopiamines A and B (**4** and **6**), which are the focus of this Article. The modifications of the indole core in the prenylated indole alkaloid family, which occur by a reaction with dimethylallyl pyrophosphate (DMAPP)[1], results in the introduction of a chromene unit as is found in (+) stephacidin A (**10**; see blue highlighted portion) or a bicyclo[2.2.2]diazaoctane core that is typical of many congeners including **11** and **12** (see red highlighted portion)[2].

Although structurally similar, the prenylated indole alkaloids display a diverse range of bioactivities including antitumor, insecticidal, anthelmintic, calmodulin-inhibition, and antibacterial properties[3]. The recent discovery of citrinadins A[4] and B[5] (**7** and **8**) and PF1270A–C[6] (**9a**–**c**) has added an unprecedented dimension to the structural motifs afforded by the *Penicillum* strains as well as raised several questions as to the biogenesis of these structurally related alkaloids. Recently, elegant syntheses of citrinadins A and B have been achieved by the groups of Martin[7] and Wood[8]. Particularly intriguing to us is a subset of this emerging subclass including citrinalins A and B (**1** and **2**) and cyclopiamines A and B (**4** and **6**), which like the citrinadins, lack the bicyclo[2.2.2]diazaoctane framework and, remarkably, possess an alkyl nitro group. Cyclopiamines A and B (**4** and **6**) were discovered first (in 1979) by Steyn and coworkers[9] from a toxinogenic strain of *P. cyclopium*, whereas citrinalins A and B (**1** and **2**), were discovered by Berlinck and coworkers in 2010 from a strain of *P. citrinum*[10]. While natural products that possess aryl nitro groups are known, those that contain aliphatic nitro groups are extremely rare[11]. As such, the citrinalins and cyclopiamines, which also possess three nitrogen atoms in chemically distinct environments, are rather unusual and therefore attractive targets for synthesis. The synthetic studies described herein have culminated in the total syntheses of *ent*-citrinalin B (*ent*-**2**; *ent*, enantiomer) and cyclopiamine B (**6**) and, along with ^{13}C feeding studies that have resulted in the isolation of two new citrinalins, provide support for a proposed biogenesis of the subset of prenylated indole alkaloids that lack the bicyclo[2.2.2]diazaoctane core.

Results and Discussion

Biosynthetic connections

As was proposed by Steyn and coworkers[9], a stimulating connection may be drawn between cyclopiamine A and B via the intermediacy of nitronate iminium ion **5** (see Figure 1). The interconversion of **4** and **6** was in fact demonstrated by Steyn et al. by heating either compound in dioxane/water or dimethylformamide (DMF) [9]. This led to a proposal that **6**,

which is the more stable of the two isomers (we have computed **6** to be 9.6 kcal/mol lower in energy as compared to **4** in a DMF solvent model, see the Supporting Information), may in fact be an isolation artifact. Given the likelihood that the citrinadins, citrinalins and cyclopiamines are all oxidative degradation products of a precursor containing a bicyclo[2.2.2]diazaoctane ring, such as marcfortine A (**11**; in the case of the citrinadins) or stephacidin A (**10**; in the case of the citrinalins and cyclopiamines), we wondered whether the citrinalins could be transformed to the cyclopiamines. On the basis of this assumption, it is particularly baffling that unlike cyclopiamines A and B, which are related by an aza-Henry (or nitro-Mannich) reaction as shown in Figure 1 (**4**⇔**6**, via **5**), citrinalin A and the originally proposed structure of citrinalin B (**3**) would be related not by the formal epimerization of the C22 stereocenter but rather by the nature of the relative configuration of the C14 carbon (highlighted in **2** and **3**). On the basis of the connection between cyclopiamine A and B as demonstrated by Steyn, we intuited that the structure of citrinalin B may be better represented by **2**. To support this proposal, we undertook a computational simulation of the ^{1}H and ^{13}C NMR spectra that would be expected for the neutral and salt forms of citrinalins A and B (see the Supporting Information for details). As has been convincingly demonstrated by Tantillo and coworkers in numerous cases, this method provides an accurate prediction of the structures of complex natural products[12]. We found that the computed and empirical data for the trifluoroacetic acid (TFA) salt form of citrinalin A is in good agreement with those reported by Berlinck and workers, who isolated these compounds. The corrected mean absolute deviation (CMAD) in the ^{1}H and ^{13}C NMR resonances is 0.21 and 2.0 ppm, respectively (largest outliers are 1.0 and 5.2 ppm, respectively). On the other hand, the computed data for the TFA salt form of **3** (the originally proposed structure of citrinalin B) differs significantly from that recorded using the naturally occurring material (CMAD = 0.45 and 2.0 ppm; largest outliers = 2.3 and 9.6 ppm for ^{1}H and ^{13}C, respectively). The best match to the reported spectral data was found to correspond to **2** in its neutral form (CMAD = 0.12 and 1.6 ppm; largest outliers = 0.38 and 4.4 ppm for ^{1}H and ^{13}C, respectively), which corroborates a potentially similar biosynthetic connection as has been established for the cyclopiamines (outlined in Figure 1). As a result, we chose to proceed with the hypothesis that **2** most likely represents the correct structure of citrinalin B. Ultimately, a reanalysis of the NMR data of citrinalin B, collected in MeOH-d_4 (see Supporting Information for details), corroborates the assignment of **2** as the true structure of citrinalin B.

Synthesis

As outlined in Figure 2, cyclopiamine B (**6**) can be obtained from the enantiomer of citrinalin B (*ent*-**2**) by employing a chromanone rearrangement to forge the tetrahydroquinolone structural moiety found in the cyclopiamines. In turn, *ent*-**2** could be taken back using an ‘indole to spirooxindole’ transform to fused hexacycle **13**. Fused indole **13** would arise from tricycle **14**, which may be prepared from diene **15**, the *tert*-butyldimethylsilyl (TBS) variant of which was first prepared by Rawal and Jewett[13], and tetrahydroindolizinone **16** (unprecedented prior to this report) that would ultimately arise from D-proline (**17**).

We initiated our synthetic studies with the *tert*-butoxycarbonyl (Boc)-protection of D-proline (Figure 3), which was followed by the reduction of the carboxylic acid group and Swern oxidation of the resulting hydroxyl to afford aldehyde **18**[14]. Alkynylative homologation of the aldehyde group of **18** using the Ohira-Bestmann method[15], followed by removal of the Boc group and acylation with 2-cyanoacetyl chloride gives alkyne **19**, which serves as a substrate for an unprecedented formal cycloisomerization likely proceeding via a metal vinylidene intermediate (generated using the method of Grotjahn)[16], anti-Markovnikov hydration, and Knoevenagel condensation to give tetrahydroindolizinone **16**. At this stage, a $SnCl_4$-catalyzed Diels-Alder [4+2] reaction[17] between **16** and diene **15** and a subsequent basic workup affords an enone (not shown), which is iodinated to yield iodoenone **20**[18]. A mild hydrolysis of the nitrile group of **20** is achieved using Pt-complex **21** under the conditions introduced by Ghaffar and Parkins[19] to afford the corresponding carboxamide, which serves as a substrate for a Hofmann rearrangement that is effected with phenyliodosylbistrifluoroacetate (PIFA) to yield carbamate **22**[20]. Suzuki cross-coupling of **22** with known boronic ester **23**[21] gives adduct **24**, which is efficiently converted to fused indole **25** using two sequential reductions – all in accord with the effective protocols established by Herzon and Myers[22].

The face selective oxygenation of C2/C3-fused indoles is a well-established route to hydroxyindolenines, which serve as precursors to the corresponding spirooxindoles[23]. As such, we envisioned the oxygenation of indole **25** (Figure 4A) as a path to the spirooxindole structural moiety found in **1**, **2**, **4** and **6**. On the basis of related precedent from Sorensen[24], Williams[25], and Martin[7] for heteroatom-directed oxygenation, we expected the carbamate group of **25** to direct oxygenation to the alpha face and provide **28**. Surprisingly, the use of the oft-employed Davis' oxaziridine[26] (**29**, 3.0 equiv) leads to **26** and trace amounts of both hydroxyindolenine **28** and spirooxindole **27** (spirooxindole **27** arises via the intermediacy of hydroxyindolenine **26)**. A survey of other oxaziridines including **30** and **31** leads, at best (using **31**), to a 1:1 ratio of the desired hydroxyindolenine **28** and both hydroxyindolenine **26** and spirooxindole **27**. Because the inherent face selectivity for the oxygenation of **25** is poor, attention was turned to the use of reagent control to achieve the desired diastereoselective oxygenation. In this regard, we were drawn to the peptide-derived catalysts developed by Miller and coworkers[27]. Following an investigation of a focused library of peptide catalysts developed in the Miller laboratory for oxygenations, **32** (Figure 4B) emerged as the superior catalyst (20 mol% loading) and provided hydroxyindolenine **28** in 83% yield from **25**. Hydroxyindolenine **28** rearranges with heating using $Sc(OTf)_3$ over 2 hours to afford pseudoindoxyl **33** (Figure 4C) instead of the desired spirooxindole. The equilibrium between pseudoindoxyls and spirooxindoles is well recognized and has been studied for the migration of C2 alkyl substituents by Borschberg[28] and recently for C2 aryl substituents by Movassaghi and coworkers[29]. However, despite prolonged heating, further rearrangement of pseudoindoxyl **33** to the desired spirooxindole was not observed. It is possible that an intramolecular hydrogen bond stabilizes pseudoindoxyl **33** toward further rearrangement (a bond distance of 2.24Å is computed for the pseudoindoxyl carbonyl group and N-H proton of the carbamate group in **33**; see the Supporting Information for more details). A possible stabilizing intramolecular hydrogen bond in **33** is supported by the observation that hydroxyindolenine **26** (prepared by oxidation of **25** with Davis' oxaziridine)

rearranges readily at room temperature in the presence of mild acid to spirooxindole **27**; a pseudoindoxyl generated from **26** would lack the analogous stabilizing hydrogen bond. However, the possibility exists that **26** proceeds to an epoxide intermediate (see **A** in inset in Figure 4C) that rearranges to **27**. The recalcitrance of pseudoindoxyl **33** to undergo further rearrangement caused us to consider alternative tactics that would produce the desired spirooxindole structural moiety of the citrinalins and cyclopiamines.

Amino compound **35** (Figure 5) was prepared on the basis of a hypothesis that an amino group or some oxidized derivative thereof (e.g., the corresponding hydroxylamine) could serve as a hydrogen bond donor to effect stereoselective oxygenation of the indole C2–C3 bond and then, by further oxidation to a nitroso or nitro group, remove the presumed intramolecular hydrogen bond that may stabilize the pseudoindoxyl form (as in **33**). It appeared reasonable that this sequence would facilitate the eventual conversion of **35** to nitro spirooxindole compound **36**. Initial experiments established that epoxidation of the chromene ring was a competing reaction that occurred under various oxygenation conditions. As such, we opted to effect a Wacker oxidation[30] of **25** to afford chromanone **34** (Figure 5), which would be advantageous as the chromanone unit is found in the citrinalins and cyclopiamines. Remarkably, treatment of **35** (following removal of the methoxycarbonyl group in **34**) with an excess of dimethyldioxirane (DMDO) (formed *in situ* from acetone and Oxone®) affords spirooxindole **36** as the major product (4:1 d.r., diastereomeric ratio) where the spiro center is as desired and the nitro group has been installed. It is possible that spirooxindole **36** arises from epoxide **B** (see inset in Figure 5) on the basis of studies by Foote and co-workers for DMDO oxidations of indoles to spirooxindoles[31]. Therefore, it is possible that the introduction of the chromanone diminishes the participatory role of the indole nitrogen lone pair leading, after rearrangement (see direction of arrow in **B**), to **36**[32]. With spirooxindole **36** in hand, what remained was a selective removal of the tertiary amide carbonyl group by reduction, which had to be accomplished in the presence of the chromanone and secondary amide carbonyl groups as well as the newly introduced nitro group. After extensive investigation, this task was effectively accomplished using a modification of a procedure developed by Borch[33] by treating **36** with a variant of Meerwein's salt (Me_3OBF_4), which likely leads to a methylated amidinium intermediate that is cleanly reduced with sodium cyanoborohydride to give *ent*-citrinalin B (*ent*-**2**) in 66% yield (79% brsm; based on recovered starting material). The spectroscopic data for the neutral form of *ent*-**2** are fully consistent with the data reported by Berlinck and coworkers for the compound believed to be citrinalin B (corroborating the computational predictions and reanalysis in MeOH-d_4), except for the sign of optical rotation, which is opposite. The structure of *ent*-**2** was unambiguously confirmed by X-ray crystallographic analysis of its HCl salt. *ent*-Citrinalin B is easily converted to cyclopiamine B (**6**) upon treatment of *ent*-**2** with sodium hydride and heating (to effect the chromanone to tetrahydroquinolone conversion) and subsequent methylation of the resulting phenol. The structure of cyclopiamine B (**6**) was also unambiguously confirmed by X-ray crystallographic analysis. Thus, the synthesis of *ent*-**2** and its conversion to **6** conclusively supports *ent*-**2** as the true structure of citrinalin B, albeit the enantiomer of the naturally occurring material.

Biosynthetic considerations

The total syntheses of *ent*-citrinalin B (*ent*-**2**; 19 steps from D-proline, 5.5% overall yield) and cyclopiamine B (**6**; 21 steps from D-proline, 4.3% overall yield) not only unambiguously establish the structures of these metabolites, but also provide possible insight into the biogenesis of these natural products (especially as to the possible formation of the cyclopiamines from the citrinalins).

The citrinalins, and in turn the cyclopiamines likely arise from a bicyclo[2.2.2]diazaoctane precursor. However, such a precursor was unknown prior to the findings that are reported herein (*vide infra*). Consistent with numerous biosynthetic studies of the prenylated indole alkaloids, the structural features of **1**, **2**, **4** and **6** suggest that tryptophan, proline and two isoprene units are biosynthetic precursors to these compounds. While no biosynthetic studies on **1** and **2** or **4** and **6** or the related citrinadins and PF1270 alkaloids has appeared, a hypothesis suggesting they are derived from bicyclo[2.2.2]diazaoctane precursors that suffer the “loss” of one diketopiperazine carbonyl group has been advanced by Kobayashi and coworkers[5]. Through the isolation of 17-hydroxycitrinalin B (**37**, Figure 6A) and more importantly citrinalin C (**38**) following a series of stable isotope labeling experiments (summarized in Figure 6B; see the Supporting Information for more details), we have now obtained support for the possible biogenesis of the citrinalins and cyclopiamines from a precursor bearing the bicyclo[2.2.2]diazaoctane moiety.

The nuclear magnetic resonance (NMR) and mass spectroscopy (MS) characterization data for **37** is fully consistent with the assigned structure. Moreover, the assigned relative configuration fully corroborates the revised structure of citrinalin B (**2**). By analogy to citrinalin B (**2**), the absolute configuration of **37** was assigned as 1*S*,14*R*,16*R*,17*R*,22*R*. 17-Hydroxycitrinalin B (**37**) was initially isolated from *P. citrinum* F53 grown in a nitrogen depleted culture medium. Stable isotope feeding studies with [U-^{13}C]anthranilic acid and [1-^{13}C]glucose gave significant ^{13}C labeling (see the Supporting Information). High levels of [U-^{13}C]ornithine were also incorporated into **37,** while additional feeding studies with [U-^{13}C]proline gave almost undetectable labeling. Ornithine is a well-known biosynthetic precursor to proline, but to our knowledge has never been reported as a efficient substrate for isotopic labeling of the putative proline-derived atoms in the biosynthesis of prenylated indole alkaloids of fungal origin bearing the bicyclo[2.2.2]diazaoctane moiety. The labeling investigations suggest that, 17-hydroxycitrinalin B (**37**) might arise from either 3-hydroxyl ornithine, 3-hydroxy proline, or by the late-stage oxygenation of the citrinalin A, B or C skeleton.

Citrinalin C (**38**), isolated as a minor component from the culture medium of *P. citrinum* F53, gives NMR and MS data (see Supporting Information, Table S4) that is fully consistent with the relative and absolute configuration illustrated for this natural product. The isolation of **38**, along with the congeners lacking the bicyclo[2.2.2]diazaoctane structural moiety from *P. citrinum* F53, lends support to a bicyclo[2.2.2]diazaoctane-containing precursor, which arises from a committed intramolecular Diels-Alder (IMDA) cycloaddition step as has been studied in detail for other congeners by Williams and Sherman[34]. In accordance with the proposal of Kobayashi, hydrolysis of the amide bridge of citrinalin C (**38**), followed by

decarboxylation, and amino group oxidation to the nitro group, as proposed in the biosynthesis of the structurally related citrinadin B[5], would then yield citrinalin A. These latter steps are the subject of current biosynthesis studies. A question that remained at this stage concerned the biogenesis of citrinalin B. On the basis of the observations of Steyn in the cyclopiamine series (see **4** → **6**, Figure 1), we anticipated that citrinalin A (**1**) might be converted to citrinalin B (**2**) via a nitronate/iminium intermediate analogous to **5**. In the event, heating a solution of a naturally occurring sample of citrinalin A (**1**) in DMF-d_7 at 100 °C for 20 hours leads to a 1:1 ratio of **1** and **2** (with complete conversion to citrinalin B (**2**) after 60 hours, see the Supporting Information, Figure S22), confirming the connection of these metabolites presumably by the same aza-Henry/nitro-Mannich epimerization sequence established for the cyclopiamines by Steyn and coworkers. However, we have observed some key differences. First, the epimerization in the citrinalin series occurs at a qualitatively slower rate (likely due to a non-productive proton transfer from the vinylogous imide N–H to the tertiary amine) and higher temperature. In addition, we have not been able to achieve any observable conversion of citrinalin B to citrinalin A even at elevated temperatures (165 °C) over prolonged periods (24 h). Our current efforts are focused on gaining a deeper understanding of these differences and exploring the biosynthetic conversion of citrinalin C to citrinalin A.

Conclusion

We have achieved the first total syntheses of the prenylated indole alkaloids *ent*-citrinalin B and cyclopiamine B. Our results secure unambiguously the identity of citrinalin B both through synthesis, a reanalysis of the naturally isolated material, and by an X-ray crystallographic study. Our studies on the isolation of metabolites from *P. citrinum* support a bicyclo[2.2.2]diazaoctane-containing metabolite such as citrinalin C (**38**) as an intermediate in the biogenesis of citrinalins A (**1**) and B (**2**) (Fig. 7). The extension of the synthetic methods reported herein to the syntheses of other prenylated indole alkaloids are ongoing and will be reported in due course.

METHODS SUMMARY

All reactions were performed under a nitrogen atmosphere using dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran, toluene, methanol, triethylamine, benzene and diethyl ether were obtained by passing the commercially available, oxygen free, solvents through activated alumina columns from GlassContour®. Dichloromethane was distilled over calcium hydride under a nitrogen atmosphere. Yields refer to materials purified using silica gel column chromatography. Full experimental details and characterization data for all new compounds (^{1}H NMR, ^{13}C NMR, mass spectrometry, infrared, R_f value), including **14–36**, **2** and **6**, appears in the Supporting Information. Crystallographic data were collected on a MicroSTAR-H APEX II (ChexStar: RUA # 1091) instrument and the Bruker SAINT and SADABS software programs were used for integrating and scaling the data, respectively. Computational analyses were conducted following conformational searches using the MMFF94 force field (Spartan'10). DFT calculations were performed with GAUSSIAN09 (B3LYP/6-31+G(d,p) level of theory). Full details are included in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

R.S. and P.G.R thank the USA National Institutes of Health (NIGMS RO1 086374) for financial support. R.S. is a Camille Dreyfus Teacher Scholar. E.V.M.M. acknowledges the National Science Foundation (USA) for a graduate fellowship (GRFP). S.R., E.F.P., and R.G.S.B are grateful to the National Counsel of Technological and Scientific Development (CNPq, Brazil, Grant 470643/2010-2) and the São Paulo Research Foundation (FAPESP, Brazil, Grant 2012/50026-3) for funding. D.E.W. and R.J.A. thank NSEPC for funding. M.W.L. and D.J.T. acknowledge support from the US National Science Foundation (CHE-0957416 and supercomputing resources through a grant from the XSEDE program: CHE-030089). S.J.M. is grateful for the NIH for support (GM096403). We thank Dr. Antonio DiPasquale (UC Berkeley) for solving the crystal structures of *ent*-**2**•HCl, **6**, **27** and **36** (supported by NIH Shared Instrumentation Grant S10 RR027172) and acknowledge the CYLView program (developed by Prof. Claude Y. Legault, Dept. of Chemistry, Université Sherbrooke) for X-ray depictions. We would like to thank Dr. Terry Lebold (currently at Jannsen Pharmaceuticals), Professors Robert M. Williams (Colorado State University) and David Sherman (University of Michigan) for helpful discussions.

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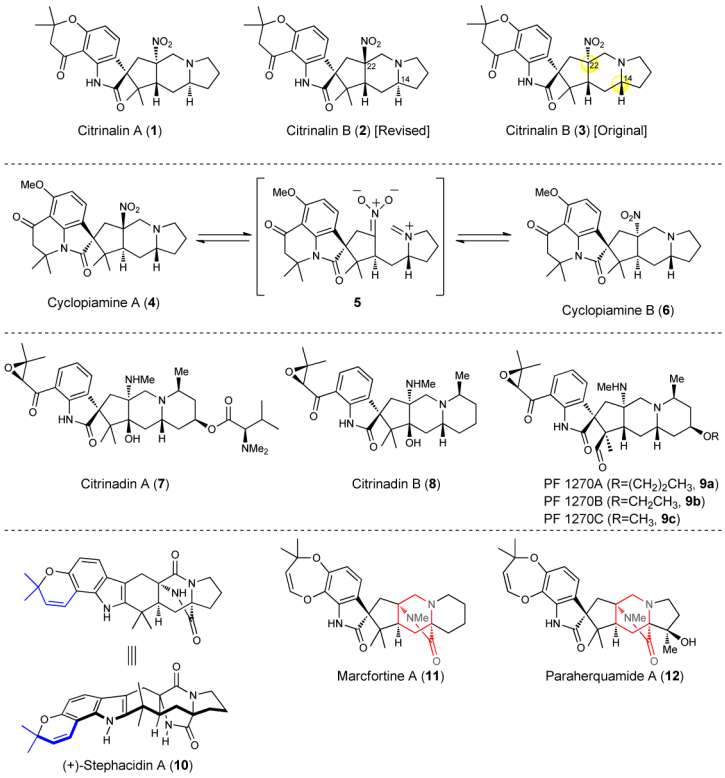

Figure 1. Selected prenylated indole alkaloids

The prenylated indole alkaloid family encompasses over 80 natural products some of which contain a bicyclo[2.2.2]diazaoctane core as in **10**, **11** and **12**. Recently, several members of this family (e.g., **1** and **4**) have emerged that do not possess this structural motif. Me, methyl.

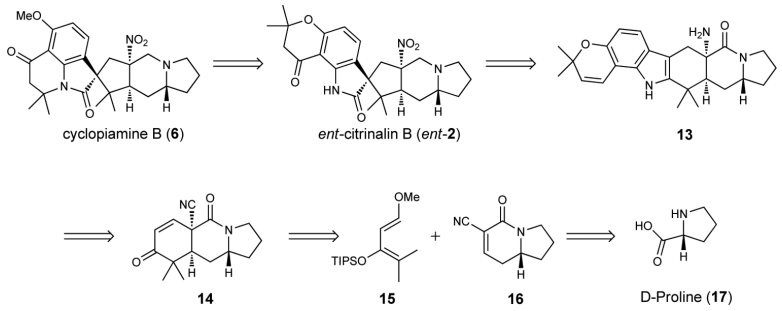

Figure 2. Retrosynthetic analysis plan for cyclopiamine B and citrinalin B

The syntheses of natural products, **2**, and **6** is expected to arise from common intermediate **13**. TIPS, triisopropylsilyl; Me, methyl.

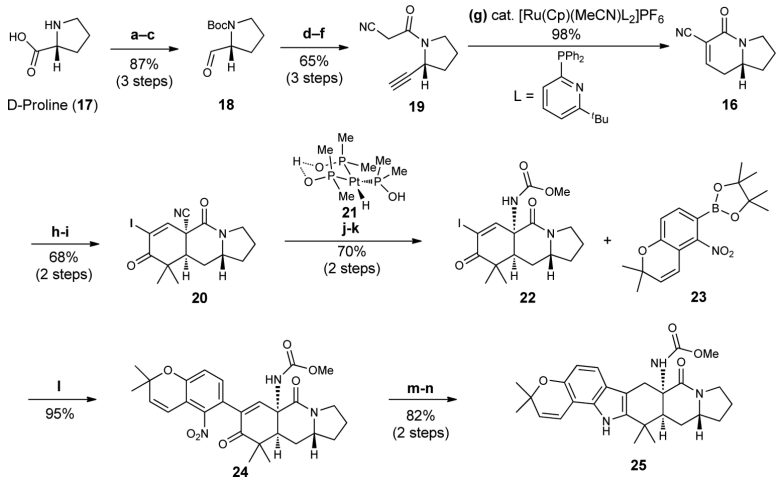

Figure 3. Preparation of fused hexacycle 25

The use of a Diels-Alder reaction involving a proline-derived indolizidinone dienophile affords a key tricycle that is advanced to hexacycle **25** by Suzuki coupling to boronic ester **23**. Reagents and conditions are as follows. **a.** di-*tert*-butyl dicarbonate **(**Boc_2O), $NaHCO_3$, H_2O/tetrahydrofuran (THF), room temperature (RT = 23 °C). **b.** BH_3•THF, THF, 0 °C to RT. **c.** $(COCl)_2$, dimethylsulfoxide (DMSO), CH_2Cl_2, diisopropylethylamine (DIPEA), − 78 °C. **d.** Dimethyl (diazomethyl)phosphonate, K_2CO_3, MeOH, 0 °C to RT. **e.** 4N HCl/ Dioxane, 0 °C to RT. **f.** 2-cyanoacetylchloride, Et_3N, CH_2Cl_2, 0 °C to RT. **g.** acetonitrile bis[2-diphenylphosphino-6-*t*-butylpyridine] cyclopentadienylruthenium(II) hexafluorophosphate (8 mol%), acetone/H_2O, 70 °C. **h. 15**, $SnCl_4$, −78 °C to −42 °C. **i.** I_2, 4-dimethylaminopyridine **(**DMAP), pyridine/CCl_4, 60 °C. **j. 21** (20 mol%), EtOH/H_2O, RT. **k.** phenyliodosylbistrifluoroacetate (PIFA), MeOH, RT. **l.** dppf$PdCl_2$ (10 mol%), K_3PO_4, dimethylformamide (DMF), 40 °C. **m.** Zn dust, NH_4Cl, HCO_2NH_4, *p*-TsOH, MeOH, RT; **n.** $NaCNBH_3$, 1 N aq. HCl, 0 °C to RT. dppf, diphenylphosphinoferrocene; Me, methyl.

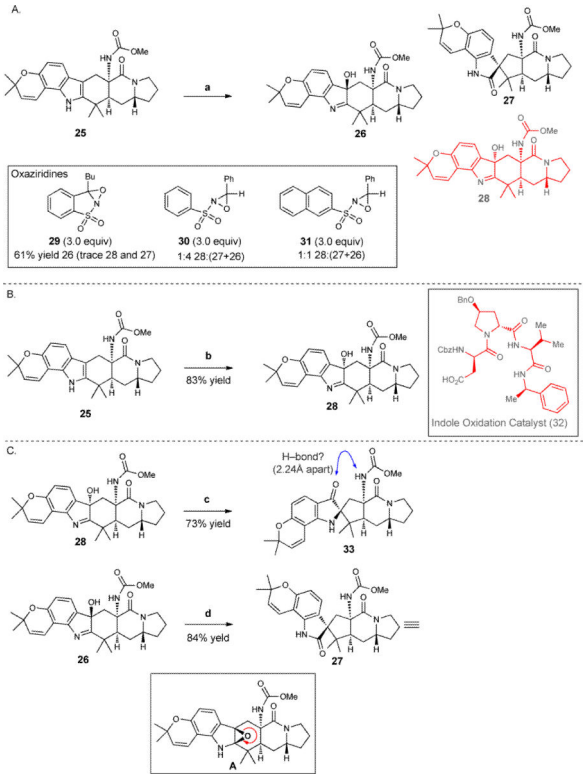

Figure 4.

A. Oxidative rearrangement studies of fused indole **25** with a range of oxaziridines leads predominantly to the undesired, epimeric, hydroxyindolenine (**26**) and spirooxindole (**27**). **Figure 4B**. Use of indole oxidation peptide catalyst **32** to effect oxidation yields the desired hydroxyindolenine **(28)**. **Figure 4C**. The desired hydroxyindolenine **28** rearranges to an undesired pseudoindoxyl (**33**) whereas the epimeric hydroxyindolenine (**26**) affords the corresponding spirooxindole (**27**). Reagents and conditions are as follows. **a.** oxaziridine (**29, 30,** or **31**), CH_2Cl_2, room temperature (RT = 23 °C). **b. 32** (20 mol%), 4-dimethylaminopyridine (DMAP), diisopropylcarbodiimide (DIC), H_2O_2, $CHCl_3$, 4 °C. **c.** $Sc(OTf)_3$, toluene, 110 °C. **d.** 23 mM HCl, CH_2Cl_2, RT. Me, methyl; Bu, butyl; Bn, benzyl; Cbz, carboxybenzyl; Ph, phenyl.

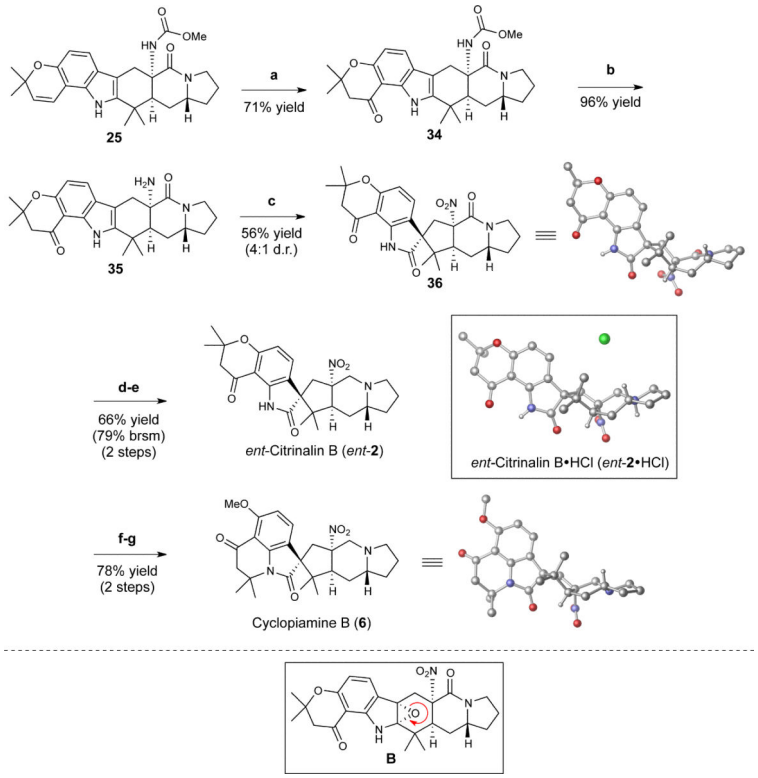

Figure 5. Completion of the syntheses of *ent*-citrinalin B and cyclopiamine B

The total synthesis of **2** and **6** required the identification of conditions that accomplished the oxidation of the amino group and spirooxindole formation in one pot as well as unique conditions to selectively reduce the tertiary amide carbonyl group. The rearrangement of *ent*-citrinalin B (**2**) to cyclopiamine B (**6**) was also demonstrated. Reagents and conditions are as follows. **a.** $Pd(OAc)_2$ (40 mol%), benzoquinone, H_2SO_4, $MeCN/H_2O$, room temperature (RT = 23 °C). **b.** Me_2S, methanesulfonic acid (MsOH), 40 °C. **c.** Oxone® (10 equiv), $NaHCO_3$, $acetone/H_2O$, °0 C to RT. **d.** Me_3OBF_4, CH_2Cl_2, 4 Å MS, 45 °C; then **e.** $NaCNBH_3$, MeOH, 0 °C. **f.** NaH, dimethylformamide (DMF), 60 °C. **g.** MeI, K_2CO_3, acetone, 60 °C. Oxone®, potassium peroxymonosulfate; MS, molecular sieves; Me, methyl; brsm, based on recovered starting material.

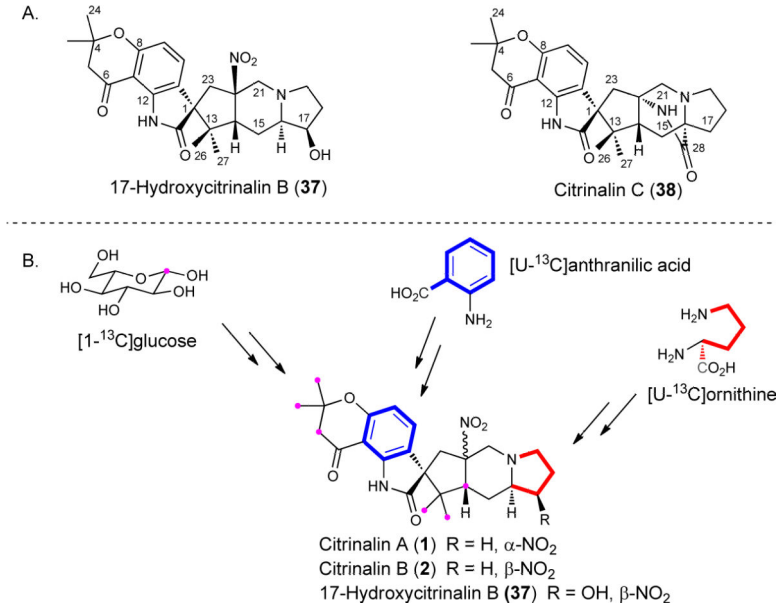

Figure 6.

A. Structures of 17-hydroxycitrinalin B and citrinalin C. Two additional citrinalins, **37** and **38**, were isolated upon refractionation and reanalysis of secondary metabolites from *P. citrinum* F53. **Figure 6B. Summary of the ^{13}C labeling studies.** ^{13}C incorporation studies of *P. citrinum* F53 reveal that glucose (pink), anthranilic acid (blue) and ornithine (red) are biosynthetic precursors to the citrinalins.

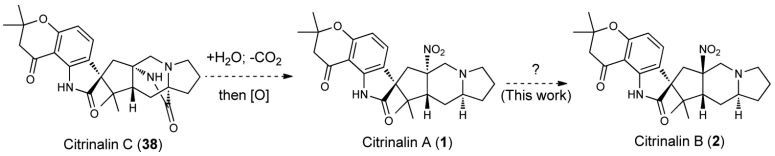

Figure 7. Biosynthetic proposal for citrinalins

Consistent with previous reports on the bicyclo[2.2.2]diazaoctane congeners, the citrinalins likely arise through an intramolecular Diels-Alder reaction to form citrinalin C (**38**), which is followed by a decarboxylation event and amine group oxidation to the nitro group.