ARTICLE

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Total synthesis and isolation of citrinalin and cyclopiamine congeners

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Many natural products that contain basic nitrogen atoms—for example alkaloids like morphine and quinine—have the potential to treat a broad range of human diseases. However, the presence of a nitrogen atom in a target molecule can complicate its chemical synthesis because of the basicity of nitrogen atoms and their susceptibility to oxidation. Obtaining such compounds by chemical synthesis can be further complicated by the presence of multiple nitrogen atoms, but it can be done by the selective introduction and removal of functional groups that mitigate basicity. Here we use such a strategy to complete the chemical syntheses of citrinalin B 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 (which contains a bicyclo[2.2.2]diazaoctane structural unit) through carbon-13 feeding studies, support the existence of a common bicyclo[2.2.2]diazaoctane-containing biogenetic precursor to these compounds, as has been proposed previously.

The prenylated indole alkaloids are an emerging class of natural products typified by the presence of an indole ring, or derivatives thereof (that is, 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 (Fig. 1, **1** and **2**) 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[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** (ref. 2) (see red highlighted portion).

Although structurally similar, the prenylated indole alkaloids display a diverse range of bioactivities including anti-tumour, insecticidal, anthelmintic, calmodulin-inhibition and antibacterial properties[3]. The recent discovery of citrinadins A (ref. 4) and B (ref. 5) (**7** and **8**) and PF1270A–PF1270C[6] (**9a**–**9c**) has added an unprecedented dimension to the structural motifs afforded by the *Penicillium* strains, and has raised several questions as to the biogenesis of these structurally related alkaloids. Recently, syntheses of citrinadins A and B have been achieved[7,8]. 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 in 1979 in a toxinogenic strain of *Penicillium cyclopium*[9], whereas citrinalins A and B (**1** and **2**) were discovered in 2010 in a strain of *Penicillium citrinum*[10]. Although natural products that possess aryl nitro groups are known, those that contain aliphatic nitro groups are extremely rare[11]. As a result, the citrinalins and cyclopiamines, which also possess three nitrogen atoms in chemically distinct environments, are unusual and are therefore attractive targets for synthesis. The synthetic studies described here 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.

Biosynthetic connections

A stimulating connection may be drawn between cyclopiamine A and B via the intermediacy of nitronate iminium ion **5** (ref. 9) (Fig. 1). The interconversion of **4** and **6** was demonstrated by heating either compound in a mixture of dioxane and water or in dimethylformamide[9] (DMF). 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^{-1} lower in energy than **4** in a DMF solvent model; see Supplementary Information), may in fact be an isolation artefact. 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 Fig. 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 stereocentre 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 cyclopiamines A and B demonstrated previously[9], 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 (Supplementary Information). As has been convincingly demonstrated 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 salt form of citrinalin A is in good agreement with those reported in ref. 10. The corrected mean absolute deviations

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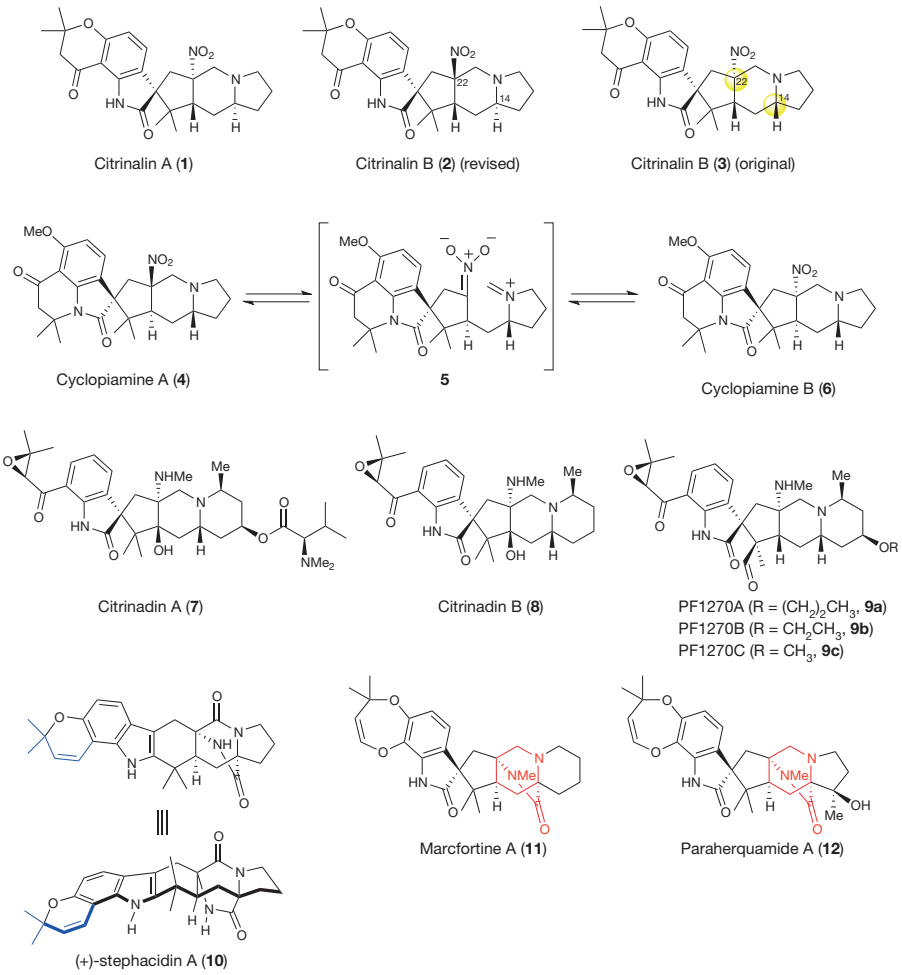

Figure 1 | Selected prenylated indole alkaloids. The prenylated indole alkaloid family encompasses over 80 natural products, some of which contain a bicyclo[2.2.2]diazaoctane core as in **10**, **11** and **12**. Recently, several members of this family (for example **1** and **4**) have emerged that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

(CMAD) in the ^{1}H and ^{13}C NMR resonances are 0.21 and 2.0 p.p.m., respectively (the largest outliers are 1.0 and 5.2 p.p.m., respectively). However, the computed data for the trifluoroacetic acid salt form of **3** (the originally proposed structure of citrinalin B) differs significantly from that recorded using the naturally occurring material (CMADs, 0.45 and 2.0 p.p.m.; largest outliers, 2.3 and 9.6 p.p.m. 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 (CMADs, 0.12 and 1.6 p.p.m.; largest outliers, 0.38 and 4.4 p.p.m. for ^{1}H and ^{13}C, respectively), which corroborates the potentially similar biosynthetic connection that has been established for the cyclopiamines (outlined in Fig. 1). As a result, we chose to proceed on the assumption that **2** most probably represents the correct structure of citrinalin B. Ultimately, a reanalysis of the NMR data of citrinalin B, collected in MeOH-d_4 (Supplementary Information), corroborates the assignment of **2** as the true structure of citrinalin B.

Synthesis

As outlined in Fig. 2, cyclopiamine B (**6**) can be obtained from the enantiomer of citrinalin B (ent-**2**) by using 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

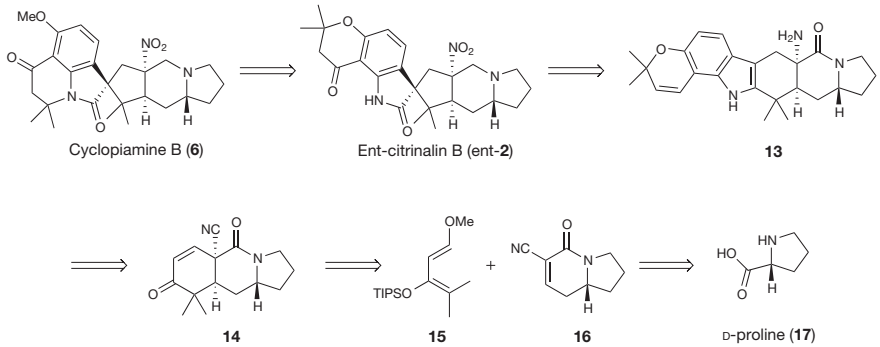

Figure 2 | Retrosynthetic analysis plan for cyclopiamine B and citrinalin B. The syntheses of natural products **2** and **6** are expected to arise from common intermediate **13**. TIPS, triisopropylsilyl.

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arise from tricycle **14**, which may be prepared from diene **15**, the *t*-butyldimethylsilyl variant of which was first prepared in ref. 13, and tetrahydroindolizinone **16**, which would ultimately arise from D-proline (**17**).

We initiated our synthetic studies with the protection of D-proline by *t*-butoxycarbonyl (Fig. 3), which was followed by the reduction of the carboxylic acid group and Swern oxidation of the resulting hydroxyl to afford aldehyde **18** (ref. 14). Alkynylative homologation of the aldehyde group of **18** using the Ohira–Bestmann method[15], followed by removal of the *t*-butoxycarbonyl group and acylation with 2-cyanoacetyl chloride gives alkyne **19**. This serves as a substrate for an unprecedented formal cycloisomerization that probably proceeds via a metal vinylidene intermediate[16], anti-Markovnikov hydration and Knoevenagel condensation to give tetrahydroindolizinone **16**. At this stage, a $SnCl_4$-catalysed Diels–Alder [4+2] reaction[17] between **16** and diene **15,** and a subsequent basic work-up, affords an enone (not shown), which is iodinated to yield iodoenone **20** (ref. 18). A mild hydrolysis of the nitrile group of **20** is achieved using Pt-complex **21** (ref. 19) to afford the corresponding carboxamide, which serves as a substrate for a Hofmann rearrangement that is effected with phenyliodosyl bistrifluoroacetate to yield carbamate **22** (ref. 20). Suzuki cross-coupling of **22** with known boronic ester **23** (ref. 21) gives adduct **24**, which is efficiently converted to fused indole **25** using two sequential reductions—all in accord with the effective protocols established in ref. 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]. We therefore reasoned that the oxygenation of indole **25** (Fig. 4a) could be a path to the spirooxindole structural moiety found in **1**, **2**, **4** and **6**. On the basis of related precedents for heteroatom-directed oxygenation[7,24,25], we expected the carbamate group of **25** to direct oxygenation to the α-face and provide **28**. Surprisingly, the use of 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 peptide-derived catalysts developed for oxygenations[27]. Following an investigation of a focused library of peptide catalysts, **32** (Fig. 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 h to afford pseudoindoxyl **33** (Fig. 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[28] and C2 aryl substituents[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** against further rearrangement (a bond distance of 2.24 Å is computed for the pseudoindoxyl carbonyl group and N–H proton of the carbamate group in **33**; see Supplementary Information). Evidence for a stabilizing intramolecular hydrogen bond in **33** comes from 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 Fig. 4c) that rearranges to **27**. The difficulty of further rearranging pseudoindoxyl **33** caused us to consider alternative approaches that would produce the desired spirooxindole structural moiety of the citrinalins and cyclopiamines.

Amino compound **35** (Fig. 5) was prepared on the assumption that an amino group, or some oxidized derivative thereof (for example 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 seemed 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**

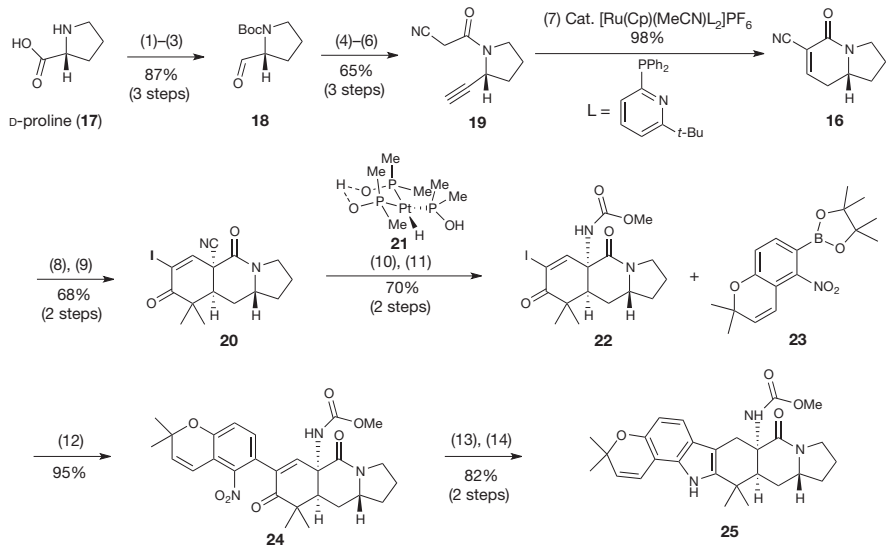

Figure 3 | Preparation of fused hexacycle 25. The use of a Diels–Alder reaction involving a proline-derived indolizidinone dienophile affords a key tricycle that is advanced to hexacycle **25** by Suzuki coupling to boronic ester **23**. Reagents and conditions are as follows: (1) di-*t*-butyl dicarbonate (Boc_2O), $NaHCO_3$, H_2O and tetrahydrofuran (THF), room temperature (RT = 23 °C); (2) BH_3•THF, THF, 0 °C to RT; (3) $(COCl)_2$, dimethylsulphoxide, CH_2Cl_2, diisopropylethylamine, −78 °C; (4) dimethyl (diazomethyl)phosphonate, K_2CO_3, MeOH, 0 °C to RT; (5) 4N HCl and dioxane, 0 °C to RT; (6) 2-cyanoacetylchloride, Et_3N, CH_2Cl_2, 0 °C to RT; (7) acetonitrile bis[2-diphenylphosphino-6-*t*-butylpyridine] cyclopentadienylruthenium(II) hexafluorophosphate (8 mol%), acetone and H_2O, 70 °C; (8) **15**, $SnCl_4$, −78 to −42 °C; (9) I_2, 4-dimethylaminopyridine, pyridine and CCl_4, 60 °C; (10) **21** (20 mol%), EtOH and H_2O, RT; (11) phenyliodosyl bistrifluoroacetate, MeOH, RT; (12) $dppfPdCl_2$ (10 mol%), K_3PO_4, DMF, 40 °C; (13) Zn dust, NH_4Cl, HCO_2NH_4, *p*-TsOH, MeOH, RT; (14) $NaCNBH_3$, 1N HCl(aq.), 0 °C to RT. dppf, (diphenylphosphino)ferrocene; Et, ethyl; *t*-Bu, *t*-Butyl; Ts, tosyl.

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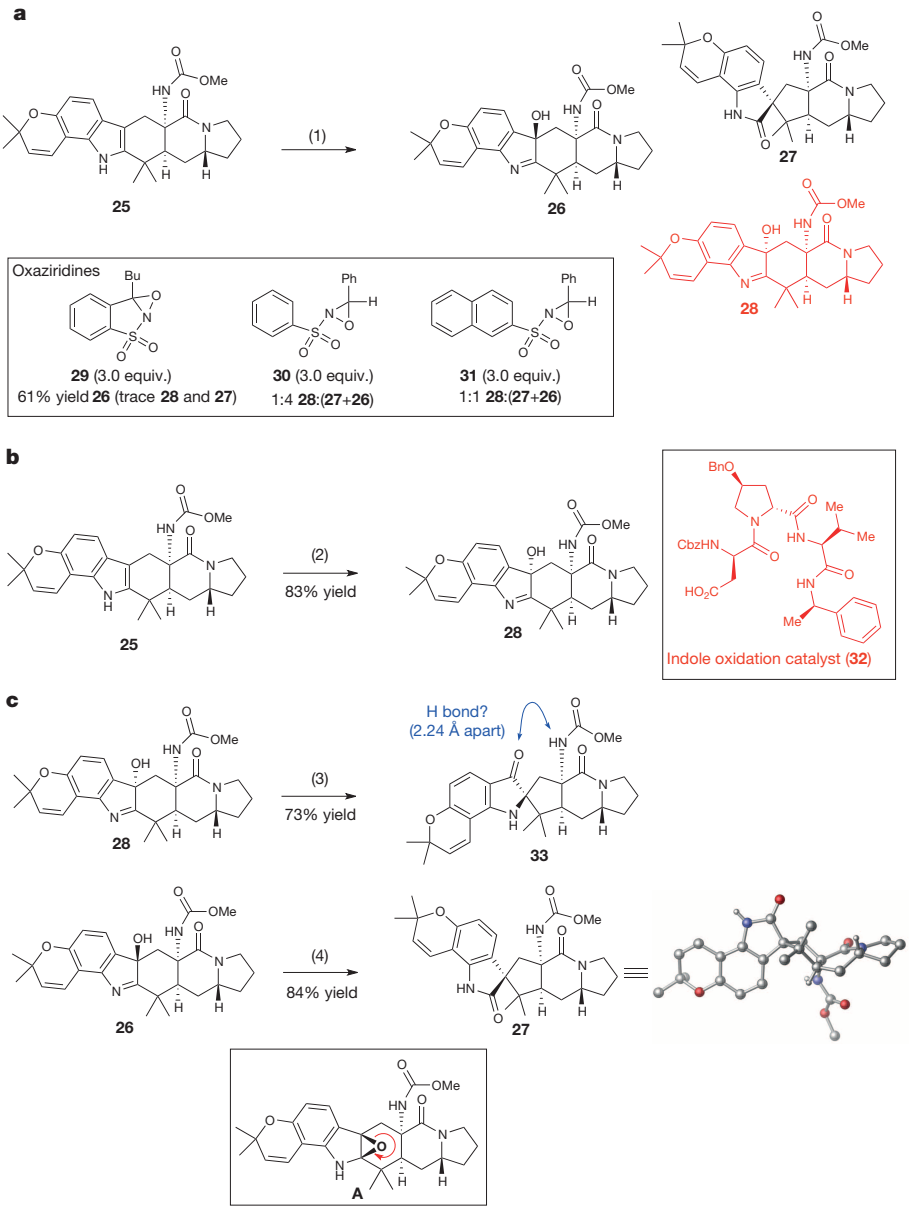

Figure 4 | Face-selective oxygenation of fused hexacycle 25. **a**, Oxidative rearrangement studies of fused indole **25** with a range of oxaziridines leads predominantly to the undesired, epimeric, hydroxyindolenine (**26**) and spirooxindole (**27**). **b**, Use of indole oxidation peptide catalyst **32** to effect oxidation yields the desired hydroxyindolenine (**28**). **c**, Hydroxyindolenine **28** rearranges to an undesired pseudoindoxyl (**33**), whereas the epimeric hydroxyindolenine (**26**) affords the corresponding spirooxindole (**27**). Reagents and conditions are as follows: (1) oxaziridine (**29**, **30**, or **31**), CH_2Cl_2, RT; (2) **32** (20 mol%), 4-dimethylaminopyridine, diisopropylcarbodiimide, H_2O_2, $CHCl_3$, 4 °C; (3) $Sc(OTf)_3$, toluene, 110 °C; (4) 23 mM HCl, CH_2Cl_2, RT. Bn, benzyl; Cbz, carboxybenzyl; Ph, phenyl.

to afford chromanone **34** (Fig. 5), which would be advantageous because 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 (formed *in situ* from acetone and Oxone) affords spirooxindole **36** as the major product (diastereomeric ratio, 4:1) where the spiro centre is as desired and the nitro group has been installed. Studies of dimethyldioxirane oxidations of indoles to spirooxindoles[31] suggest that spirooxindole **36** might arise from epoxide **B** (Fig. 5, inset). 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**. 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 known procedure[32] by treating **36** with a variant of Meerwein's salt (Me_3OBF_4), which probably leads to a methylated amidinium intermediate that is cleanly reduced with sodium cyanoborohydride to give ent-citrinalin B (ent-**2**) in 66% yield (79% based on recovered starting material). The spectroscopic data for the neutral form of ent-**2** are fully consistent with previous data reported for the compound believed to be citrinalin B (ref. 10; 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**) on treatment of ent-**2** with sodium hydride and heating (to effect the conversion of chromanone to tetrahydroquinolone) and subsequent methylation of the resulting phenol. The structure of cyclopiamine B (**6**) was also unambiguously confirmed by X-ray

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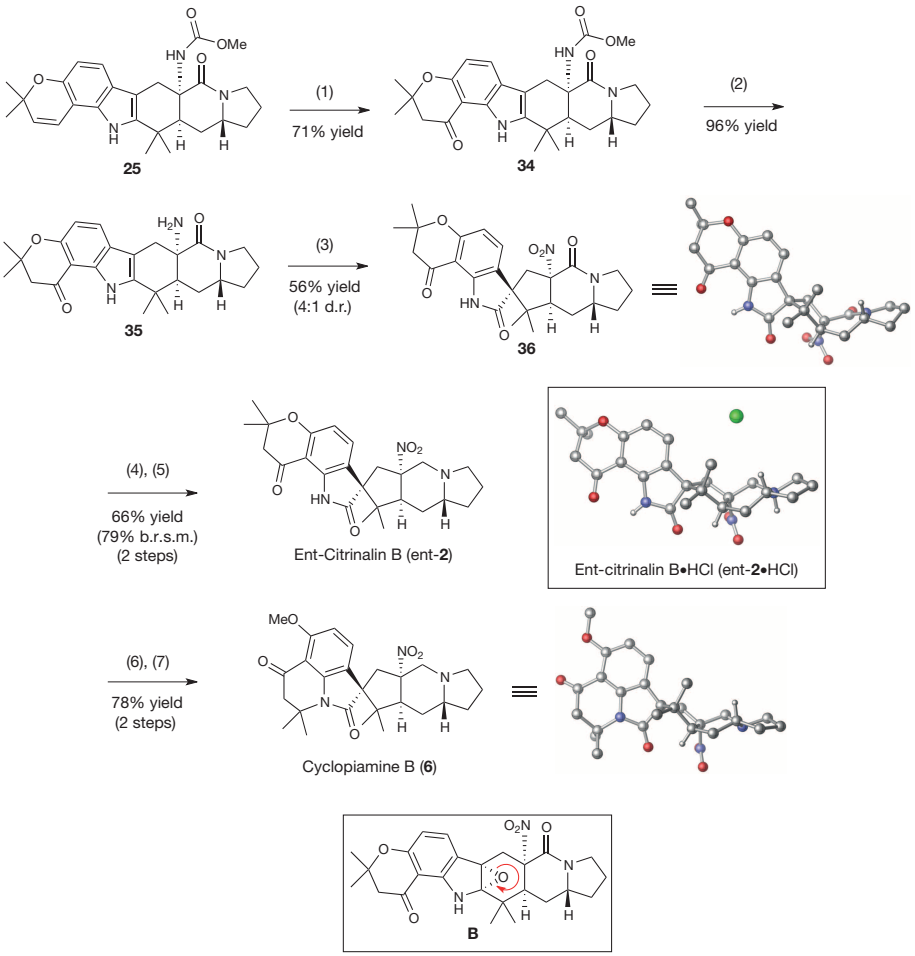

Figure 5 | Completion of the syntheses of ent-citrinalin B and cyclopiamine B. The total syntheses of **2** and **6** required the identification of conditions that accomplished the oxidation of the amino group and spirooxindole formation in one pot as well as unique conditions for the selective reduction of 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: (1) $Pd(OAc)_2$ (40 mol%), benzoquinone, H_2SO_4, MeCN and H_2O, RT; (2) Me_2S, methanesulphonic acid, 40 °C; (3) Oxone (10 equiv.), $NaHCO_3$, acetone and H_2O, 0 °C to RT; (4) Me_3OBF_4, CH_2Cl_2, 4 Å molecular sieves, 45 °C; (5) $NaCNBH_3$, MeOH, 0 °C; (6) NaH, DMF, 60 °C; (7) MeI, K_2CO_3, acetone, 60 °C. b.r.s.m., based on recovered starting material; d.r., diastereomeric ratio; Oxone, potassium peroxymonosulphate.

crystallographic analysis. Thus, the synthesis of ent-**2** and its conversion to **6** show that ent-**2** is 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, probably arise from a bicyclo[2.2.2]diazaoctane precursor. However, such a precursor was unknown before the findings that are reported herein (see below). 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. Although no biosynthetic studies on **1** and **2** or **4** and **6** or the related citrinadins and PF1270 alkaloids has appeared, it has been suggested that they are derived from bicyclo[2.2.2]diazaoctane precursors that suffer the 'loss' of one diketopiperazine carbonyl group[5]. Through the isolation of 17-hydroxycitrinalin B (**37**; Fig. 6a) and, more importantly, citrinalin C (**38**) following a series of stable isotope labelling experiments (summarized in Fig. 6b; see Supplementary Information), 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 NMR and mass spectroscopy 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**).

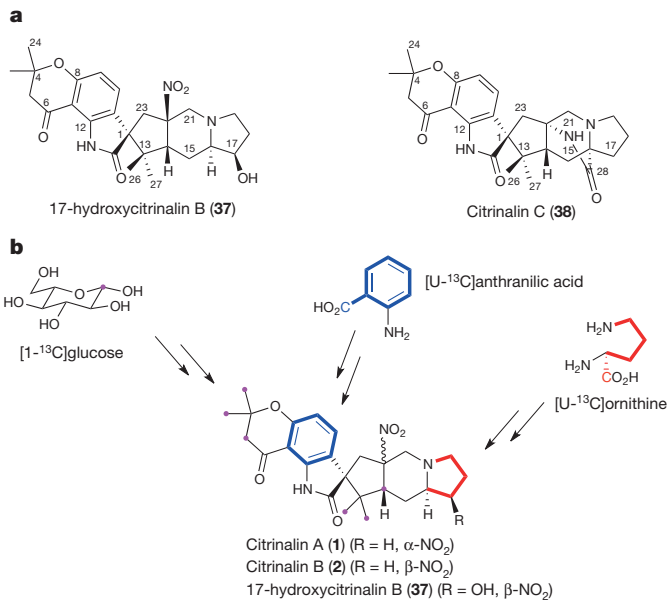

Figure 6 | Isolation of two new citrinalins and ^{13}C labelling studies. **a**, Structures of 17-hydroxycitrinalin B and citrinalin C. Two additional citrinalins, **37** and **38**, were isolated on refractionation and reanalysis of secondary metabolites from *P. citrinum* F53. **b**, Summary of the ^{13}C labelling 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.

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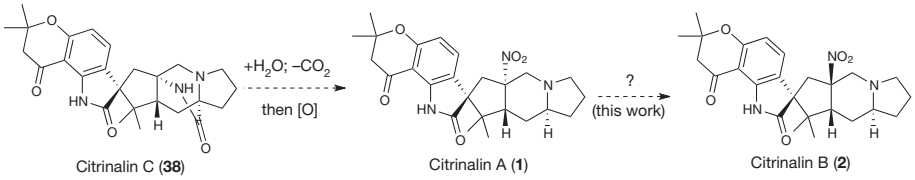

Figure 7 | Biosynthetic proposal for citrinalins. Consistent with previous reports on the bicyclo[2.2.2]diazaoctane congeners, the citrinalins probably arise through an intramolecular Diels–Alder reaction to form citrinalin C (**38**), which is followed by a decarboxylation event and amine-group oxidation to the nitro group.

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 labelling (Supplementary Information). High levels of [U-^{13}C]ornithine were also incorporated into **37,** and additional feeding studies with [U-^{13}C]proline gave almost undetectable labelling. Ornithine is a well-known biosynthetic precursor to proline, but to our knowledge it has never been reported as an efficient substrate for isotopic labelling 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 labelling 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 mass spectroscopic data (Supplementary Table 4) 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 cycloaddition step such as that studied in detail for other congeners[33]. Hydrolysis of the amide bridge of citrinalin C (**38**; Fig. 7), 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 observations of the cyclopiamine series[9] (see **4** → **6** in Fig. 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 h leads to a 1:1 ratio of **1** and **2** (with complete conversion to citrinalin B (**2**) after 60 h; see Supplementary Fig. 22), confirming the connection of these metabolites presumably by the same aza-Henry or nitro-Mannich epimerization sequence established for the cyclopiamines[9]. However, we have observed some key differences. First, the epimerization in the citrinalin series occurs at a qualitatively lower rate (probably owing 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 reported the total syntheses of the prenylated indole alkaloids ent-citrinalin B and cyclopiamine B. Our results unambiguously identify citrinalin B through synthesis, a reanalysis of the naturally isolated material and an X-ray crystallographic study. Our studies on the isolation of metabolites from *P. citrinum* suggest that a bicyclo[2.2.2]diazaoctane-containing metabolite such as citrinalin C (**38**) is an intermediate in the biogenesis of citrinalins A (**1**) and B (**2**) (Fig. 7). The extension of the synthetic methods reported here to the syntheses of other prenylated indole alkaloids is 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**, appear in Supplementary 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. The CYLVIEW program (developed by C. Y. Legault) was used for X-ray depictions. Computational analyses were conducted following conformational searches using the MMFF94 force field (SPARTAN'10). Density functional theory calculations were performed with GAUSSIAN09 (B3LYP/6-31+G(d,p) theory level). Full details are included in Supplementary Information.

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Author Contributions R.S. conceived and directed the synthetic aspects of the research, and wrote 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 carried out 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 done by P.G.-R. The computational NMR studies of **1**, **2** and **3** were designed and performed 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 A sample of the *P. citrinum* strain F53 is deposited at the Brazilian Collection of Environmental and Industrial Microorganisms under the accession code CBMAI 1186. Crystallographic data for crystal structures ent-**2**•HCl, **6**, **27** and **36** have been deposited at the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk) under accession codes CCDC 984477, CCDC 984478, CCDC 984480 and CCDC 984479, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper.
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