Synthetic studies toward citrinadin A: construction of the pentacyclic core

Monica E McCallum, Genessa M Smith, Takanori Matsumaru, Ke Kong, John A Enquist Jr and John L Wood

This manuscript describes the preparation of an advanced intermediate toward the total synthesis of citrinadin A, featuring a [3+2] cycloaddition employing in situ generation of the dipole.

The Journal of Antibiotics (2016) 69, 331–336; doi:10.1038/ja.2016.25; published online 9 March 2016

INTRODUCTION

In the early 2000's, Kobayashi and coworkers1 reported the isolation of two secondary metabolites, citrinadin A and B, from the marine fungus Penicillium citrinum N059. Structural determination efforts by Kobayashi revealed that the two congeners possess the same complex spirooxindole-containing pentacyclic core and differ only by an N,N-dimethylvaline appendage resident on citrinadin A (cf. 1 and 2, Figure 1, top).

The interesting structural features of these compounds have led several groups to begin exploring their total syntheses. Recently our efforts and those of the Martin group led to completed syntheses of citrinadins A and B, respectively. These efforts independently led to the conclusion that the structures assigned by Kobayashi were sound in terms of connectivity, but required revision at the stereochemical level.2-5 As illustrated in Figure 1 (bottom), the reassigned structures of citrinadins A and B differ at the stereogenic centers residing within the pentacyclic core. Herein, we report synthetic efforts toward citrinadin A (3) that have now resulted in the stereoselective preparation of its core structure.

RESULTS AND DISCUSSION

Citrinadin A possesses several synthetically challenging features, including an epoxy ketone side chain and a rare N,N-dimethylvaline ester. We envisioned that both of these moieties would be installed at a late stage, a maneuver that was particularly important for the delicate epoxy ketone. Thus, from a retrosynthetic perspective, citrinadin A was seen as arising from 5, wherein an aryl bromide is poised for installation of the epoxy ketone side chain, and a masked alcohol will enable installation of the ester through a Mitsunobu reaction (Scheme 1). Epoxide 5 would be available from pentacycle 6 via reductive cleavage of the isoxazolidine N–O bond and subsequent quinolizidine formation. The isoxazolidine 6 would result from the intermolecular [3+2] cycloaddition of enone 7 and nitrone 8. The requisite enone is the same as that employed in our previous synthesis of citrinadin B, whereas the nitrone was expected to arise from known diol 11 (vide infra).

During our initial studies towards citrinadin A,6 we attempted to preform the nitrone before cycloaddition. Although this approach was successful in our synthesis of citrinadin B, under similar conditions6 performed poorly. After considerable experimentation, we eventually turned to a strategy that employed in situ generation of the requisite nitrone.6 As illustrated retrosynthetically in Scheme 2, delivery of the nitrone would follow from desilylative cyclization of tert-butyl dimethylsilyl (TBS)-protected oxime 10, which in turn would arise from the known diol 11.7

In the forward sense, known diol 11 was converted to the known lactone 12 using a sequence previously reported by Nakamura et al.7, which involved exposure to pyridinium p-toluenesulfonate (PPTS) in refluxing dichloroethane, followed by tert-butyl diphenyloxirlyn (TBDDS) protection (Scheme 3) (nitrone precursor 10 was prepared using racemic ethyl-3-hydroxybutyrate in lieu of (S)-3-hydroxybutanoate as described in Nakamura et al.7). Subsequent treatment of 12 with diisobutyl aluminum hydride (DIBAL-H) furnished the corresponding lactol 13 as a 1:1 mixture of diastereomers which, upon exposure to hydroxylamine hydrochloride, underwent smooth conversion to oxime 14 as a 1:1 mixture of E and Z isomers. Conversion of 14 into a substrate suitable for desilylative cyclization required initial TBS protection of the oxime alcohol and activation of the remaining secondary alcohol through tosylation.

Having prepared racemic nitrone precursor 10, we began to explore its ability to undergo cycloaddition with the previously prepared racemic enone 7. As alluded to above, attempts to employ the preformed nitrone met with limited success. Thus, we began investigating generation of the nitrone in situ and, after an exhaustive screen of fluorous sources, solvents, and ratios of 7–10, we discovered that tetrabutylammonium difluorotriphenylsicate (TBAT) in benzene with three equivalents of 10 gave the best overall result (Scheme 4).6

We were pleased to learn that unlike the nitrone cycloaddition used en route to citrinadin B, which required 9 days, the reaction of 7 with...
Figure 1 The citrinadins.

Scheme 1 Retrosynthetic analysis of citrinadin A.

Scheme 2 Retrosynthesis of nitrone 9.

Scheme 3 Synthesis of the nitrone precursor.
10 furnished the desired cycloadduct in just 24 h. Although 15b was the minor diastereomer, we have demonstrated in preliminary studies that use of enantioenriched 7,5 gives a diastereomeric ratio (d.r.) of 3:2, whereas use of enantioenriched 7 and enantioenriched 10, derived from commercially available chiral ethyl-3-hydroxybutyrate, gives a d.r. of 2:1. Fortunately, 15a and 15b were separable via flash column chromatography, thus in these early studies, we were able to continue advancing the desired cycloadduct (15b) towards citrinadin A.

Toward this end, 15b was treated with trimethylsulfoxonium iodide and sodium hydride (Scheme 5). The derived Corey–Chaykovsky adduct (16) was stereoselectively produced in good yield.9,10 Subsequent exposure of 16 to trimethylsilyl chloride and NaI promoted intramolecular attack on the epoxide by the isoxazolidine nitrogen to furnish an ammonium salt (17), which was then reduced with zinc in AcOH to give diol 18, thereby completing construction of the core ring system.11 This began setting the stage for eventual incorporation of the angular nitrogen atom. Thus, the least hindered alcohol in 18 was activated for displacement by treating with MsCl and Et3N. Subsequent exposure of the derived mesylate to K2CO3 promoted the formation of key intermediate 19, wherein the ring-fusion epoxide stands ready to mediate incorporation of the final nitrogen.

**CONCLUSION**

In summary, we have prepared a late-stage intermediate en route to citrinadin A with an approach that employs an intermolecular [3+2] nitrene cycloaddition. The latter was found to best proceed under conditions wherein the nitrene is produced *in situ* and, although the cycloaddition was performed with racemic substrates, its stereocchemical outcome clearly indicates that enantioenriched substrates will selectively deliver material required for preparation of the natural product.

---

**Scheme 4** [3+2] Nitrene cycloaddition.

---

**Scheme 5** Completion of the core ring system.
product. These efforts have also illustrated that the derived cyclode- 
duct can be advanced to an intermediate (19) that contains all of the 
functional groups required for conversion to citrinadin A. The aryl 
brodime will allow for attachment of the epoxy ketone, removal of the 
TBDBP group will set the stage for introduction of the valine ester via 
a Mitsunobu reaction, and opening of the ring-fusion epoxide will 
provide access to the requisite anti relationship between the methy- 
lanine and alcohol substriments. Work toward these ends is underway 
and will be reported in due course.

EXPERIMENTAL PROCEDURE

General

Unless otherwise noted, all reactions have been carried out with distilled 
and degassed solvents under an atmosphere of dry N₂ in oven- 
(135 °C) or flame-dried glassware with standard vacuum-line techniques. Triethylamine, diso-
propyamine and methanol were dried over calcium hydride and freshly 
distilled. Benzene, tetrahydrofuran, methylene chloride, toluene, acetonitrile and 
diethyl ether were dried using a solvent purification system manufactured 
by 5G Water U.S.A., LLC (Nashua, NH, USA) as follows: tetrahydrofuran, 
diethyl ether, acetonitrile and methylene chloride were passed through 
two packed columns of neutral alumina, whereas benzene and toluene were passed 
through a column of alumina and a column of Q5. All other commercially 
available reagents were used as received.

Unless otherwise stated, all reactions were monitored by TLC using glass-
backed extra hard layer, 60 Å plates (Indicator F-254, 250 μm, Silicycle, Ville de 
Québec, QC, Canada). Column or flash chromatography was performed with 
the indicated solvents using Silicycle SiliaFlash P60 (230–400 mesh) silica gel as 
the stationary phase. All melting points were obtained on a Gallenkamp 
capillary melting point apparatus (model: MPD350.BM2.1, Sanyo Electric Co., 
Osaka, Japan) and are uncorrected. IR spectra were obtained using a Nicolet Avatar 320 FTIR (Thermo Electron Corporation, Madison, WI, USA) or Bruker Tensor 27 FTIR (Bruker Optics Inc., Billerica, MA, USA).¹H and 
¹³C NMR spectra were recorded on a Varian Inova 300, Varian Inova 400, 
Varian Inova 400 autosampler, or Varian Inova 300 spectrometer (Varian, Inc., 
Palo Alto, CA, USA). Chemical shifts (δ) are reported in p.p.m. relative to 
internal residual solvent peaks from indicated deuterated solvents. Coupling 
constants (J) are reported in Hertz (Hz) and are rounded to the nearest 0.1 Hz.

Multiplicities are defined as: s=singlet, d=doublet, t=triplet, q=quartet, 
m=multiplet, dd=doublet of doublets, dt=doublet of triplets, ddd=doublet of doublets, 
dd=doublet of doublets, and br=broad, app=apparent, par=partial. HRMS were performed at the Central 
Instrument Facility by Donald L. Dick of Colorado State University.

Lactol 13. Lactone 12 (3.2 g, 8.7 mmol) in dichloromethane (90 ml) was 
cooled to −78 °C for the addition of disobutylaluminum hydride solution 
(1 M in hexanes, 9.6 ml, 9.6 mmol). This was stirred cold for 2 h at which time 
the reaction was quenched at −78 °C with methanol (9 ml) and allowed to 
warm to room temperature. The resulting reaction solution was vigorously 
stirred with an added solution of Rochelle’s salt (9 g in 100 ml H₂O) for 1 h. 
Following separation of the layers, the aqueous portion was extracted with 
diethyl ether, and the combined organics were washed with brine, dried over 
sodium sulfate (Na₂SO₄) and concentrated to provide lactol 13 as a 1:1 mixture 
of anomers (3.2 g, 100% yield), which was used in the next step without further 
purification.¹H NMR (400 MHz, CDCl₃) δ 7.71–7.68 (m, 8H), 7.46–7.36 (m, 
2H), 5.30 (br, t, 1H), 4.50 (dd, J = 9.7, 6.6, 2.1 Hz, 1H), 4.22 (m, 1H), 3.91 
(2H), 3.79 (t, J = 10.9, 4.8 Hz, 1H), 3.31 (ddq, J = 11.6, 5.9, 1.9 Hz, 1H), 3.01 
(t, J = 2.6 Hz, 1H), 2.09 (dd, J = 12.2, 4.4, 2.1 Hz, 1H), 1.98 
(ddd, J = 12.8, 4.7, 1.7 Hz, 1H), 1.76 (m, 2H), 1.60 (dd, J = 12.9, 11.0, 3.6, 
2.0 Hz, 1H), 1.48–1.26 (m, 3H), 1.19 (d, J = 6.2 Hz, 1H), 1.11 (d, J = 6.3 Hz, 
3H), 1.08 (s, 9H), 1.07 (s, 9H);¹³C NMR (100 MHz, CDCl₃) δ 135.83, 134.60, 
134.48, 134.24, 134.13, 129.84, 129.82, 129.70, 129.69, 127.76, 127.74, 127.67, 
127.65, 94.39, 92.93, 68.28, 68.16, 65.14, 64.16, 43.30, 42.61, 42.29, 39.51, 
27.07, 27.01, 21.51, 21.27, 20.19, 19.19; IR (thin film): 3398 (br, m), 2923 (m), 
2858 (m), 1428 (m), 1112 (s), 702 (s) cm⁻¹; HRMS (ESI) Calcd. for 
C₂₃H₂₈NaO₅Si [M+Na]⁺: 393.1858. Found: 393.1862.
1177 (s), 1111 (m), 919 (m), 703 (m) cm$^{-1}$; HRMS (ESI) Calcd. for C$_{33}$H$_{32}$NO$_5$Si$^+$ [M+H]: 564.3105. Found: 564.3113.

**Isoxazolines 15a and 15b.** To a solution of the nitrene precursor 10 (118 mg, 0.18 mmol) in benzene (900 µl) was added MgSO$_4$ (43 mg, 0.36 mmol) and the enone 7 (245 mg, 0.06 mmol). Lastly, tetrabutylammonium difluorotriphenylsilicate (97 mg, 0.18 mmol) was added in two portions. This stirred at ambient temperature overnight (17 h). At that time, the heterogeneous mixture was diluted with dichloromethane (1 ml) and filtered through a celite plug. The filtrate was directly adsorbed onto silica gel and purified by flash chromatography (5% → 8% → 12% → 30% ethyl acetate/hexanes) to provide two isoxazoline diastereomers (39 mg total, 84% yield, 15a+15b=dr. 7:3), the desired isoxazoline 15b (12 mg) and the undesired diastereomer 15a (27 mg), both as white foams. Isoxazolines 15a: $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.70 (m, $J=1.5$ Hz, 4H), 7.57 (dd, $J=7.6, 0.9$ Hz, 1H), 7.44–7.27 (m, 10H), 7.23 (d, $J=7.2$ Hz, 2H). 6.91 (t, $J=7.9$ Hz, 1H), 5.42 (br m, 2H), 3.98 (br s, 1H), 3.79 (br m, 1H), 3.42 (br m, 2H), 2.90 (s, 2H), 2.44 (br m, 1H), 2.02 (d, $J=1.4$ Hz, 1H), 1.91 (d, $J=1.4$ Hz, 1H), 1.55 (s, 3H), 1.25 (m, 1H), 1.13 (m, 12H), 1.01 (s, 3H), 0.97 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.35, 19.24, 19.90, 20.62, 22.44, 27.10, 32.31, 38.16, 39.59, 43.70, 44.96, 48.24, 48.67, 55.01, 61.28, 65.67, 77.76, 93.73, 102.10, 123.49, 125.97, 126.57, 127.20, 127.78, 128.65, 128.90, 128.96, 133.98, 134.02, 134.60, 136.01, 136.06, 137.18, 140.04, 184.50, 213.66; IR (thin film) 2930 (1751), 1715 (1710), 1450 (m), 1104 (s), 733 (cm$^{-1}$); HRMS (ESI) Calcd. for C$_{35}$H$_{35}$BrN$_2$O$_4$Si [M+H]+: 779.2723. Found: 777.2703.

**Spiraeoxide 16.** To a solution of trimethylsulfoxonium iodide (145.3 mg, 0.66mmol) in dimethylsulfoxide (2.2 ml) was added NaH (60% in mineral oil, 26.4 mg, 0.66 mmol). After stirring at room temperature for 4 h, the resulting homogeneous solution was added to a solution of the epoxide 15a (8 mg, 0.009 mmol) in dimethylsulfoxide (2.2 ml) and MsCl and triethylamine were again added and allowed to stir for 2 h, after which equal amounts of MsCl and triethylamine were again added and the reaction was stirred in a sealed flask for 2 more days. This was then filtered through a pad of Celite, rinsed with dichloromethane and concentrated in vacuo. The resulting yellowish oil was dissolved in dichloromethane, washed with NaHCO$_3$ (sat. aq. sohn.), and the aqueous portion extracted with ethyl acetate/dichloromethane. The combined organic layers were concentrated and purified by flash chromatography (basic Al$_2$O$_3$, 0% → 1% → 2% methanol/dichloromethane) to provide the desired product 16 as a clear oil (5 mg, 71% yield). $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.67 (m, 4H), 7.48 (dd, $J=7.5, 1.1$ Hz, 4H), 7.39 (m, 6H), 7.32 (dd, $J=8.1, 1.1$ Hz, 4H), 7.28 (m, 2H), 7.21 (m, 3H), 6.93 (dd, $J=8.1, 5.1$ Hz, 1H), 5.36 (s, 2H), 4.90 (d, $J=2.7$ Hz, 1H), 4.31 (b s, 1H), 3.92 (tt, $J=11.0, 4.5$ Hz, 1H), 3.23 (dd, $J=10.2, 1.0$ Hz, 2H), 2.98 (m, 1H), 2.75 (tt, $J=11.1, 2.8$ Hz, 1H), 2.47 (d, $J=14.4$ Hz, 1H), 2.22 (d, $J=10.2$ Hz, 1H), 2.02 (d, $J=14.4$ Hz, 1H), 1.82 (dd, $J=12.2, 4.5, 2.4$ Hz, 1H), 1.74 (td, $J=11.7, 4.7$ Hz, 1H), 1.63–1.57 (m, 2H), 1.45 (dd, $J=13.0, 3.4$ Hz, 1H), 1.32 (m, 1H), 1.22 (s, 3H), 1.05 (s, 9H), 0.87 (s, 3H), 0.75 (s, $J=6.9$ Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 183.51, 140.15, 137.31, 136.57, 135.89, 135.84, 134.76, 134.34, 133.70, 129.74, 129.72, 128.66, 127.71, 126.78, 127.27, 126.87, 125.64, 120.01, 82.23, 81.97, 77.36, 66.16, 61.05, 56.35, 55.56, 52.49, 46.38, 46.06, 45.22, 43.74, 41.96, 36.13, 29.86, 27.19, 27.14, 21.66, 19.27, 11.79; IR (thin film): 3390 (br), 3350 (w), 2959, 2929, 2856, 1686 (s), 1450, 1339, 1106, 1064, 734, 701 (cm$^{-1}$); HRMS (ESI) Calcd. for C$_{36}$H$_{37}$BrN$_2$O$_4$Si [M+H]+: 795.3036. Found: 793.3028.

**Ring-fusion epoxide 19.** To a solution of the diol 18 (116 mg, 0.141 mmol) in dichloromethane (3.0 ml) at room temperature was added MsCl (32.5 µl, 0.423 mmol), dropwise, and let stir for 5 min. To the resulting clear yellow solution was added triethylamine (0.2 ml, 1.41 mmol), dropwise, to give a dark orange, clear solution that was allowed to stir for 2 h, after which equal amounts of MsCl and triethylamine were again added and allowed to stir for 3 h. Methanol (3.0 ml) was added, followed by K$_2$CO$_3$ (0.083 g, 0.60 mmol), to give a cloudy orange reaction mixture. After 16 h, the reaction was quenched with NaHCO$_3$ (sat. aq. sohn., 1 ml), extracted with dichloromethane/ethyl acetate (2:1), and the combined organic layers were dried with brine, dried over Na$_2$SO$_4$ and concentrated in vacuo. The resulting residue was dried on silica gel and purified via flash column chromatography (5 → 10 → 20 → 30 → 50% ethyl acetate/hexanes+1% triethylamine ea.) to furnish the desired epoxide as an off-white foam (83.0 mg, 76% yield) and recovered starting material (29 mg). Note: To ensure reproducible $^1$H NMR data, product was taken up in 10% methanol/ dichloromethane, passed through a plug of basic alumina with 10% methanol/ dichloromethane as eluent and concentrated in vacuo. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.67 (ddd, $J=8.1, 4.2, 1.5$ Hz, 4H), 7.45–7.40 (m, 2H), 7.40–7.35
(m, 4H), 7.31 (dd, J = 8.2, 1.1 Hz, 1H), 7.25 (d, J = 6.6 Hz, 1H), 7.23 – 7.14 (m, 5H), 7.08 (dd, J = 7.6, 1.2 Hz, 1H), 6.84 (t, J = 7.8 Hz, 1H), 5.31 (s, 2H), 3.90 (tt, J = 10.4, 4.4 Hz, 1H), 3.16 – 3.06 (m, 2H), 3.02 (dq, J = 7.3, 3.9, 3.5 Hz, 1H), 2.60 (d, J = 14.6 Hz, 1H), 2.58 – 2.52 (m, 1H), 2.19 (d, J = 14.6 Hz, 1H), 1.92 – 1.77 (m, 3H), 1.69 (ddd, J = 12.2, 10.5, 4.5 Hz, 1H), 1.64 – 1.57 (m, 1H), 1.33 (dt, J = 12.7, 10.6 Hz, 1H), 1.06 (s, 3H), 0.94 (s, 3H), 0.71 (d, J = 6.6 Hz, 3H). 13CN M R(126M H z ,CDCl3) δ 179.47, 140.70, 138.06, 137.99, 135.87, 135.82, 134.61, 134.46, 134.41, 129.73, 129.16, 128.41, 128.38, 128.35, 127.71, 127.68, 127.66, 127.07, 126.98, 125.43, 125.27, 122.51, 101.86, 70.14, 65.93, 65.55, 59.07, 52.47, 49.54, 47.93, 44.97, 44.66, 43.10, 42.15, 41.89, 31.89, 31.66, 27.11, 25.95, 21.60, 20.34, 19.23, 11.44. IR (thin film): 3069 (w), 2931, 2856, 2247 (w), 1724, 1461, 1333, 1109 (s), 1070, 732, 702, 511 cm⁻¹; HRMS (ESI) Calcd. for C₄₅H₅₂BrN₂O₃Si [M+H+2]: 777.2931. Found: 777.2910.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
We thank Amgen, Bristol-Myers Squibb and the NSF (CHE-1058292) for their financial support. We are grateful for generous funding from Baylor University, the Welch Foundation (Chair, AA-006) and the Cancer Research & Prevention Institute of Texas (CPRIT, R1309). MEM is grateful for the support of the NSF GRFP (2013156410). TM is grateful to the Uehara Foundation; Professor Toshiaki Sunazuka, Professor Satoshi Ōmura and the Kitasato Institute for postdoctoral support. JAE thanks the NIH (GM095076) for a postdoctoral fellowship. Dr Chris Rithner, Don Heyse, and Don Dick are acknowledged for their assistance in obtaining spectroscopic data.

1 Tsuda, M. et al. A novel pentacyclic alkaloid from marine-derived fungus Penicillium citrinum. Org. Lett. 6, 3087–3089 (2004).
2 Bian, Z., Marvin, C. C. & Martin, S. F. Enantioselective total synthesis of (−)-citrinadin A and revision of its stereochemical structure. J. Am. Chem. Soc. 135, 10886–10889 (2013).
3 Kong, K. et al. An enantioselective total synthesis and stereochemical revision of (+)-citrinadin B. J. Am. Chem. Soc. 135, 10890–10893 (2013).
4 Bian, Z., Marvin, C. C., Pettersson, M. & Martin, S. F. Enantioselective total syntheses of citrinadins A and B. Stereochemical revision of their assigned structures. J. Am. Chem. Soc. 136, 14184–14192 (2014).
5 Matsunaru, T. et al. Synthetic studies toward the citrinadins: enantioselective preparation of an advanced spirooxindole intermediate. Tetrahedron 70, 4089–4093 (2014).
6 Smith, G. S. Progress Toward the Total Synthesis of the Citrinadins (Ph.D. Thesis, Colorado State University, Fort Collins, CO, 2012).
7 Nakamura, R., Tanino, K. & Miyashita, M. Stereoselective synthesis of premisakinolide A, the monomeric counterpart of the marine 40-membered dimeric macrolide misakinolide A. Org. Lett. 7, 2929–2932 (2005).
8 Chackalamannil, S. & Wang, Y. An enantioselective route to trans-2,6-disubstituted piperidines. Tetrahedron 53, 11203 (1997).
9 Corey, E. J. & Chaykovsky, M. Dimethyloxosulfonium methylide ((CH₃)₂SOCH₂) and dimethylsulfonium methylide ((CH₃)₂SCH₂). Formation and application to organic synthesis. J. Am. Chem. Soc. 87, 1353–1364 (1965).
10 Aggarwal, V. K. & Winn, C. L. Catalytic, asymmetric sulfur ylide-mediated epoxidation of carbonyl compounds: scope, selectivity, and applications in synthesis. Acc. Chem. Res. 37, 611–620 (2004).
11 Caputo, R., Mangoni, L., Neri, G. & Palumbo, G. Direct conversion of oxiranes to alkenes by chlorotrimethylsilane and sodium iodide. Tetrahedron Lett. 22, 3551–3552 (1981).