Total Synthesis of the Proposed Structure for Spirofungin B: A Reassignment of the Stereochemistry

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ABSTRACT



The total synthesis of the proposed structure for spirofungin B (2) is described. The data for the synthetic material did not compare with that for the natural product leading to the conclusion that the structure 2 assigned for spirofungin B is incorrect. Analysis of the NMR data reported for spirofungins A and B as well as related spiroketals allowed for the reassignment of the stereochemistry of spirofungin B to be that corresponding to 15-*epi*-spirofungin A (27).

The spirofungins A (1) and B (2) were isolated from a *Streptomyces* strain Tü 4113 collected in the Otway National Park, Australia.¹ Compounds 1 and 2 possess a similar 6,6-spiroketal core to reveromycin A (3)² and have the same C1–C10 triene acid and C20–24 diene acid segments; however, there is no succinate half ester at C18 and the C18 substituent is a methyl group. The spirofungins show high inhibition activity against yeast and moderate activity against some fungi with a MIC of 15 mg mL⁻¹ against *Candida albicans*.¹

Compounds (1) and (2) were isolated as a \sim 4:1 mixture, respectively. Although they possessed slightly different retention times on reverse phase HPLC, they were not separated and the structural elucidation was conducted on this mixture. The structure of the major isomer spirofungin A (1) was assigned using various NMR techniques with the minor component spirofungin B (2) identified as a diastereoisomer of 1, epimeric at both C18 and C19. The absolute configurations of 1 and 2 are suggested by analogy to the reveromycins.



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It was proposed¹ that the spiroketal core of **1** possessed the conformation **A** as depicted in Figure 1, with the oxygens (maximum anomeric stabilization) and the C19 diene acid side chain in axial orientations while the C18 methyl group is equatorial. In compound **2**, the C19 side chain is now equatorial while the C18 methyl group is oriented axial as shown in proposed conformer **B**. This was supported by the vicinal coupling constant measured between H19 and H18, the C18 change in chemical shift, and the ROE observed between H19 and H11.

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The natural occurrence of spirofungin B (2) is curious since it possesses a different absolute configuration at C19 to that from both 1 and the related reveromycins. Several groups have reported approaches^{3,4} to the spiroketal systems of spirofungins A (1) and B (2) as well as reveromycin A (3)^{5,6,7} which has also succumbed to total synthesis.^{8,9} In this paper, we report the total synthesis of the structure 2 proposed for the minor component spirofungin B which led to the reassignment of the stereochemistry of this natural product.

A brief retrosynthetic analysis of 2 is shown in Scheme 1. The key bond disconnections are similar to those utilized



for our total synthesis of reveromycin B.¹⁰ The C2–C3 and C8–C9 bonds could be formed via stabilized Wittig reactions while the C4–C5 bond might be formed by a reagent-controlled asymmetric *syn*-aldol reaction.¹¹ Finally, the C21–C22 bond could be installed using a Stille cross-coupling reaction.¹² This leads to the key spiroketal intermediate **4** which

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could be constructed from a coupling¹³ between the anion derived from alkyne **5** and Weinreb amide **6** followed by alkyne saturation and acid induced deprotection and spiroketal formation.¹⁴

The route to the nominal structure for spirofungin B began with the known optically pure alcohol 7^{10} (Scheme 2). Sily-



^{*a*} Reagents and conditions: (a) TESCl, imidazole, DMF, rt; (b) cat. OSO₄, H₂O/THF, then NaIO₄; (c) Ph₃P=CHCON(OMe)Me, CH₂Cl₂; (d) H₂, Pd/C; (e) Ph₃P, CBr₄, CH₂Cl₂, then *n*-BuLi (2 equiv), THF, -78 °C; (f) *n*-BuLi, THF, -78 to 0 °C, cool to -78 °C then add **6** in THF; (g) Lindlar catalyst, H₂, EtOAc, rt; (h) PPTS, MeOH/CH₂Cl₂; (i) TBSCl, imidazole, DMF, rt.

lation of **7** gave ether **8** which was smoothly converted into Weinreb amide **6** by oxidative alkene cleavage, Wittig extension, and hydrogenation.

Coupling fragment **5** was synthesized from known alcohol 9^{15} by protection, alkene cleavage, and Corey–Fuchs¹⁶ alkyne formation. Treatment of the alkyne **5** with *n*-BuLi followed by addition of the amide **6** and subsequent complete saturation using H₂ and Lindlar catalyst gave the corresponding ketone **10** in excellent yield without any benzyl group removal. Compound **10** was subjected to acid deprotection and spiroketalization by treatment with PPTS in MeOH to give **4** as the only spiroisomer. Some of the primary TBS group was cleaved in this sequence but this was easily reinstalled by subjection of the crude product to resilvlation. With the spiroketal fragment **4** in hand we next appended the C3–C10 side chain as outlined in Scheme 3.

Debenzylation of **4** followed by oxidation¹⁷ and homologation using the Bestmann protocol¹⁸ gave alkyne **11**. TBAF induced desilylation then afforded the alcohol **12** which fortunately was crystalline and an X-ray crystal structure¹⁹

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^{*a*} Reagents and conditions: (a) H₂, Pd/C, EtOAc; (b) Dess-Martin periodinane, CH₂Cl₂, rt; (c) MeOH, K₂CO₃, (MeO)₂P(O)C(N₂)-C(O)Me; (d) TBAF, THF, rt; (e) Ph₃P=C(Me)CHO, toulene, reflux; (f) Ph₃P=CHCO₂Me, toulene, reflux; (g) DiBALH, CH₂Cl₂, -78 °C; (h) Oxazolidine-2-thione **15**, Sn(OTf)₂, *N*-ethylpiperidine, -40 °C, CH₂Cl₂, then add **14**, -78 °C; (i) NaBH₄, THF/H₂O.

was obtained for this intermediate confirming the stereochemistry (Figure 2). Oxidation of alcohol **12** and dual Wittig



Figure 2. X-ray structure of spiroketal 12.

extensions gave the ester 13 in good yield. Adjustment of the oxidation level in 13 gave aldehyde 14 which underwent an asymmetric aldol reaction with the tin enolate derived from oxazolidine-2-thione 15.^{10,11} This sequence of reactions which provided *syn*-aldol adduct 16 was high yielding and easily conducted on large scale. Auxiliary removal by treatment of 16 with NaBH₄ in wet THF gave diol 17.

The final steps to compound **2** are outlined in Scheme 4. Conversion of alkyne **17** into vinyl stannane **18** using Pd



^{*a*} Reagents and conditions: (a) Bu₃SnH, Pd(Ph₃P)₂Cl₂, CH₂Cl₂, O °C; (b) iodide **19**, Pd₂(dba)₃, TFP, NMP, 55 °C, 5 h; (c) TBSCl, imidazole, DMF; (d) HF•pyrdine/pyrdine, THF, 0 °C; (e) Dess–Martin periodinane, CH₂Cl₂, rt; (f) Ph₃P=CHCO₂Tmse, CH₂Cl₂, rt. (g) TBAF, DMF, rt, 24h.

catalysis²⁰ proceeded in good yield. The stannane was then cross-coupled²¹ with the known iodide 19^{10} (Tmse = trimethylsilylethyl) to cleanly provide ester 20 with the C20-C24 side chain fully installed.

Protective group manipulation gave 21 which upon oxidation and Wittig reaction provided the protected precursor 22. Exposure of 22 to excess TBAF in DMF caused global deprotection to give the nominal structure for spirofungin B (2) which was purified by reverse phase HPLC. Unfortunately, the physical data obtained for synthetic 2 did not compare with that reported for spirofungin B.¹ Furthermore, the ¹H NMR spectrum of an authentic spirofungin mixture measured on the same instrument did not match our synthesized material. In particular, the chemical shift for H19 in the natural product (4.76 ppm) was vastly different to that found for compound 2 (4.29 ppm). In addition to other shift differences in the ¹H NMR spectrum, the chemical shift for C15 in the ¹³C NMR spectrum of 2 was also measurably different to the literature value (Natural spirofungin B: 97.7 ppm. Synthetic 2: 96.2 ppm.) These differences lead to the obvious conclusion that the structure proposed for spirofungin B (2) is incorrect.

An investigation of the literature revealed a possible alternative for the structure of spirofungin B. In their synthesis of the spiroketal fragment of spirofungin A, Shimizu and co-workers⁴ obtained a mixture of spiroketals **23** and **24** (ratio 1.53:1) isomeric at the spiro center via a thermodynamic approach similar to that utilized above (Figure 3).

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Figure 3. Related spiroketals (spirofungin numbering).

This clearly demonstrates that isomerization of the spirofungin A spiroketal is possible under acidic conditions (due to the axial orientation of the C19 substituent) to afford a spiroketal with one less anomeric stabilization.²² The chemical shift for the H19 in the preferred isomer is upfield from the corresponding shift in the minor isomer where the axial H19 is deshielded by the axial oxygen²³ and the opposite change in chemical shift is evident for H11 (Figure 3). Comparable chemical shift differences are also observed for H11 and H19 in spirofungins A and B themselves (see A and \mathbf{B}' , respectively, in Figure 4). Another example can be found in the synthesis of the spiroketal fragment of reveromycin A (3). Theodorakis and co-workers⁶ also obtained a mixture of spiroketal isomers 25 and 26 in a ratio of 1.5:1 respectively by acid induced spiroketalization. The chemical shift differences (Figure 3) for the H11 and H19 protons for each isomer are similar to those cited above. In addition, the C15 chemical shifts for each isomer differ in a manner similar to that for spirofungin A (95.7 ppm) verses B (97.7 ppm). This is consistent with the trend of a slight downfield shift of the spiro carbon in going from ax-ax to ax-eq oxygen orientations.²⁴ An NOE was also observed between H19 and H11 which was also seen in spirofungin B (Figure 1).



Figure 4.

The results cited above strongly suggest that the structure for spirofungin B is in fact 15-*epi*-spirofungin A (**27**) (Figure 4). The absolute configurations at C18 (*S*) and C19 (*R*) in spirofungin B are identical to that for spirofungin A (**1**) while the spiro carbon is epimeric (15*R*). Spirofungin B therefore has the conformation **B'** as shown which is consistent with the NMR data presented. It is not unreasonable to suggest that biosynthetically, spirofungin A (**1**) could give rise to **27** by simple spiroketal isomerization in analogy to the spiroisomers of the pectenotoxins.²⁵ Efforts toward the synthesis of spirofungin A (**1**) and its C15 epimer **27** are in progress and will be reported in due course.

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Supporting Information Available: Characterization data for compounds **2**, **4**–**6**, **10**, **12**, **13**, **16**, **17**, and **20**–**22** as well as NMR spectra of **2** and authentic spirofungins A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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