ORGANIC LETTERS

2007
Vol. 9, No. 2
239−242

Ascospiroketals A and B, Unprecedented Cycloethers from the Marine-Derived Fungus *Ascochyta salicorniae*

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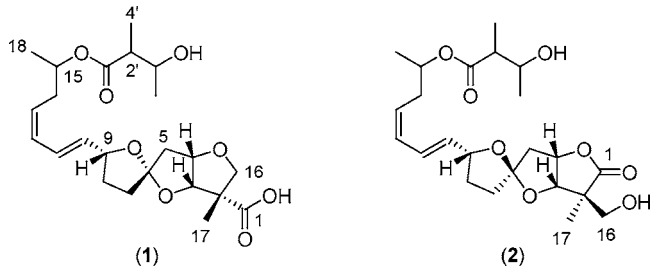
Received November 2, 2006

ABSTRACT



Chemical investigation of the marine-derived fungus *Ascochyta salicorniae* led to the isolation of two novel natural products, ascospiroketals A (1) and B (2). From a biosynthetic standpoint, the compounds possess new ring systems.

The marine-derived fungus *Ascochyta salicorniae* is distinguished by the exceptional diversity of its polyketide secondary metabolism. Initial chemical studies with this strain led to the isolation of ascosalipyrrolidinone A, an unusually substituted tetramic acid derivative with potent antiplasmodial activity, together with ascosalipyrone, a metabolite of pure polyketide origin.[1] After being cultivated under different culture conditions (OSMAC approach[2]) the strain yielded two new epimeric lactones, ascolactones A and B, as well as the known compounds ascochitine, ascochital, and hyalopyrone. Elucidation of the absolute stereostructures of the ascolactones was accomplished by a combination of analytical and chemical methods with quantum-chemical CD calculations.[3,4] All metabolites were evaluated in a panel of protein phosphatases. Ascochitine showed moderate inhibition of the mycobacterial protein tyrosine phosphatase B (MPtpB) which is regarded as a new drug target in the treatment of tuberculosis. Here we wish to report on the isolation and structure elucidation of two additional compounds from the strain. Even though the tricyclic core of ascospiroketals A (**1**) and B (**2**) bears some resemblance to cephalosporolides E and F (**3** and **4**), the structural type is unprecedented.

^{1}H NMR guided chromatographic separation using ODS VLC, Sephadex LH-20 gel filtration, and ODS HPLC yielded the new compounds **1** and **2**.

The molecular formula of **1** was determined as $C_{23}H_{34}O_8$ by high-resolution EI-MS. From the ^{13}C NMR and IR spectroscopic data, it was evident that four of the seven

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10.1021/ol0626802 CCC: $37.00
Published on Web 12/22/2006

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds **1** and **2** in $(CD_3)_2CO$ (δ in ppm, J in Hz)

		1			**2**		
position	DEPT	δC	δH	NOESY	δC	δH	NOESY
1	qC	173.6			180.1		
2	qC	55.2			51.6		
3	CH	91.2	4.38 d (3.5)	4, 5, 7[a], 17	84.1	4.60 d (4.4)	4, 5a, 16, 17
4	CH	83.0	4.73 q (3.5)	3, 5, 16b[a], 17	82.2	5.10 ddd (2.2, 4.4, 6.9)	3, 3′, 5a, 5b[a], 15[a], 16a
5	CH_2	43.2	2.16 d (3.5)	3[a], 4, 7, 16a[a]	42.9	a: 2.58 dd (6.9, 14.8)	3[a], 4
						b: 2.27 dd (2.2, 14.8)	7
6	qC	117.0			116.0		
7	CH_2	38.1	1.94 m		35.6	2.10 m	
8	CH_2	32.2	a: 2.05 m	9	31.8	a: 2.21 m	7, 9
			b: 1.70 m	10		b: 1.67 m	7, 9, 10
9	CH	81.9	4.46 q (8.2)	5[a], 7[a], 8a, 8b[a], 10, 11	79.6	4.55 q (6.9)	8a, 8b[a], 10, 11
10	CH	138.2	5.76 dd (8.2, 15.1)	8b, 9, 12, 16a	135.4	5.71 dd (6.9, 15.1)	8b, 9, 12
11	CH	126.0	6.45 dd (11.0, 15.1)	9, 12, 14, 15[a], 16a[a], 18[a]	126.7	6.58 dd (11.0, 15.1)	9, 12, 14, 15[a]
12	CH	131.5	6.06 t (11.0)	2′, 10, 13	131.2	6.11 t (11.0)	2′[a], 10, 11[a], 13
13	CH	126.9	5.38 td (11.0, 7.9)	12, 14, 15, 18	127.6	5.45 m	12, 14, 15, 18
14	CH_2	34.4	a: 2.47 m	11, 13, 15, 18	34.5	a: 2.52 m	11, 13[a], 18
			b: 2.43 m	11, 13, 15, 18		b: 2.42 m	11, 13, 15, 18
15	CH	70.7	4.90 sxt (6.3)	2′, 11, 13, 14, 18	70.6	4.91 sxt (6.3)	2′, 13, 14a, 14b, 18
16	CH_2	73.5	a: 4.63 d (7.9)	10, 11	68.0	a: 3.67 d (10.4)	3, 4, 17
			b: 3.47 d (7.9)	4[a], 17		b: 3.64 d (10.4)	3, 4, 17
17	CH_3	21.0	1.25 s	3, 4, 16b	14.0	1.09 s	3, 16a[a], 16b
18	CH_3	19.7	1.20 d (6.3)	14a, 15	19.8	1.22 d (6.3)	15
1′	qC	175.0			175.0		
2′	CH	48.1	2.34 p (6.6)	3′, 4′, 5′, 12, 15	48.1	2.33 p (6.6)	3′, 4′, 5′, 10, 12, 15
3′	CH	68.8	3.89 p (6.6)	2′, 4′, 5′	68.9	3.89 p (6.6)	2′, 4′, 5′
4′	CH_3	12.8	1.14 d (6.6)	2′, 3′	12.9	1.14 d (6.6)	2′, 3′
5′	CH_3	21.6	1.13 d (6.6)	2′, 3′	21.7	1.13 d (6.6)	2′, 3′

[a] Weak signal.

elements of unsaturation could be attributed to two olefinic (δ 126.0 CH, 126.9 CH, 131.5 CH, 138.2 CH) and two carbonyl functions (δ 173.6 qC, δ 175.0 qC). The ^{13}C NMR spectrum further revealed signals for four methyl, five methylene, six methine, and two quaternary carbon atoms (Table 1). One methylene (C-16) and five methine groups (C-3, C-4, C-9, C-15, C-3′) resonated between 65 and 95 ppm. These shifts are characteristic of oxygen-bearing carbons and pointed, together with a signal representative of a spiroketal function (C-6, δ 117.0), to a polyether with three oxygen-bearing heterocycles and at least one free hydroxyl function (ν 3416 cm^{-1}). This is consistent with the three elements of unsaturation which still had to be accounted for. After assignment of all protons to their directly attached carbon atoms via 2D HSQC, three independent spin systems (fragments A−C, Figure 1) could be deduced from the $^{1}H-^{1}H$ COSY spectrum. Thus, cross-peaks between the resonances for H-3 and H-4 and H_2-5 indicated these protons and their respective carbons to be adjacent. In addition, the spectrum revealed continuous chains of coupling from H_2-7 to H_3-18 and from H_3-4′ to H_3-5′, respectively.

Fragments A−C (Figure 1) were connected using HMBC correlations. The connectivity of fragments A and B via the spirocenter C-6 was implied by cross-peaks between the resonances for H_2-5 and C-6 and between those for H_2-7 and C-6. Coupling between CH-15 and C-1′ and between H-2′ and C-1′ showed that fragment C was part of a 3-hydroxy-2-methylbutanoic acid moiety, linked to fragment B via an ester bond. Further evidence for this side chain moiety came from EI-MS data. Thus, the fragment *m*/*z* 320 indicated loss of 3-hydroxy-2-methylbutanoic acid through McLafferty-type rearrangement.

Couplings between H-4 and C-16, between H-3 and C-6, and between H-9 and C-6 showed all of the cyclic structures to be tetrahydro furan rings. Thus, all three heterocycles of the molecule were established from the HMBC data. From couplings between H_3-17 and carbon atoms C-1, C-2, C-3, and C-16, the substitution pattern of the outer ring was deduced.

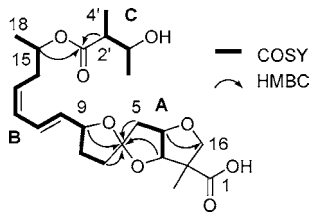


Figure 1. $^{1}H-^{1}H$ COSY and selected $^{1}H-^{13}C$ long-range correlations of **1**.

The geometry of the conjugated diene has been established from the $^1H-^1H$ coupling constants. Thus, Δ^{10} ($J_{10,11} = 15.1$ Hz) is *E* configured whereas $J_{12,13} = 11.0$ Hz suggests a *Z* configuration for Δ^{12}.

The relative configuration of the cyclic part of the molecule was established by extensive one- and two-dimensional NOE spectroscopy. Irradiation at the resonance frequency of H-4 caused enhancement of the resonances for H-3, H-16b, and H-17 and established that all these atoms were located on the same side of the ring system (Figure 2). Irradiation at the resonance frequency of H-3 supported these results and additionally gave enhancement of the resonances for H_2-7. Further evidence for the relative configuration of the spirocenter came from the NOE observed between H-10 and H-16a. This interaction can only take place if the relative configuration is as depicted in Figure 2. As irradiation of the resonance frequency of H-9 caused enhancement of the resonance for H_2-5, it was evident that these atoms are at the same side of the molecule. Thus the relative configuration of the tricyclic part of ascospiroketal A (**1**) is best described as being 2*S**,3*S**,4*S**,6*S**,9*R**.

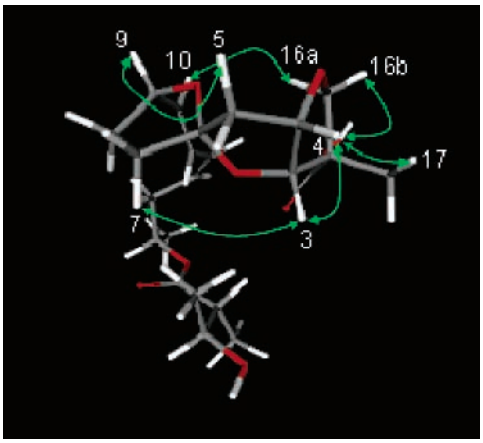


Figure 2. 3D drawing of **1** with selected diagnostic NOEs (green arrows). Configuration at C-2′, C-3′, and C-15 proposed.

From accurate mass measurement, ascospiroketal B (**2**) was found to have the same molecular formula as ascospiroketal A (**1**). Moreover, the spectroscopic data were very similar to those of compound **1**, suggesting a related planar structure (Table 1). Major differences in the ^{13}C NMR chemical shifts only appeared for the signals for C-1, C-2, CH-3, CH-4, CH_2-16, and CH_3-17, showing this outer ring to be the moiety of difference. The ^1H NMR spectrum of **2** displayed much less difference between the chemical shifts for H-16a and H-16b, suggesting that CH_2-16 was not part of the rigid ring system as in **1**. Detailed analysis of the HMBC data indeed revealed the presence of a γ-butyrolactone in **2** instead of a tetrahydro furan moiety. Optimization of the HMBC experiment for 5 Hz couplings gave a cross-peak between the resonances for H-4 and C-1, and no coupling between H-4 and C-16 was observed.

The relative configuration of the fixed part of the molecule was established by 2D NOESY and selective gradient NOESY experiments (Figure 3). Irradiation at the resonance frequency of H-4 caused enhancement of the resonances for H-3 and H-16a and established that these atoms were located on the same side of the ring system. Irradiation at the resonance frequency of H-5a gave enhancement of the resonance for H-3 suggesting the former also pointed toward the same direction, and no NOE of H-3 was detected after irradiation of H-5b. Irradiation of the resonance frequency of H-5b gave enhancement of the resonance associated with H_2-7, allowing the relative configuration of the spirocenter to be deduced. Irradiation at the resonance frequency of H-4 caused a weak enhancement of the resonance for H-15. This dipolar coupling can only occur if the side chain is oriented as shown in Figure 3. On the basis of these data, the relative configuration of the cyclic moiety of **2** is suggested to be 2*S**,3*S**,4*S**,6*R**,9*R**.

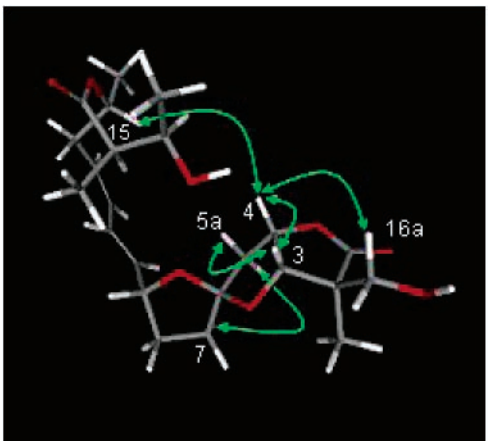
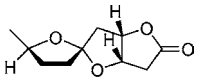


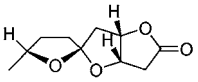
Figure 3. 3D drawing of **2** with selected diagnostic NOEs (green arrows). Configuration at C-2′, C-3′, and C-15 proposed.

The configuration of the side chain stereocenters is not easily established and remained unclear. An attempt was made to determine the absolute configuration at C-2′ and C-3′ via hydrolysis of the ester and comparison of the reaction product with standard substances. Attempts to separate the standards of 3-hydroxy-2-methylbutanoic acid by chiral GC-MS and chiral HPLC proved unsuccessful. $^3J_{H2'H3'}$ (6.4 Hz) was determined in a homonuclear decoupling experiment. The medium-sized coupling constant points to the existence of a conformational equilibrium. Therefore, analysis of the relative configuration of these stereocenters via the *J*-based approach was not successful either.

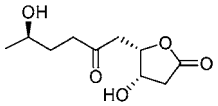
The cyclic cores of the compounds are extremely rare in nature. The core structure of **1** has been reported for some brominated acetogenins from *Laurencia* species. As these compounds are assumed to arise from straight-chain C_{15} precursors, they are biosynthetically not related to the ascospiroketals.[5,6] The core of **2**, bearing a γ-butyrolactone instead of a tetrahydro furan moiety has been reported only



Cephalosporolide E (**3**)



Cephalosporolide F (**4**)



(+)-Bassianolone (**5**)

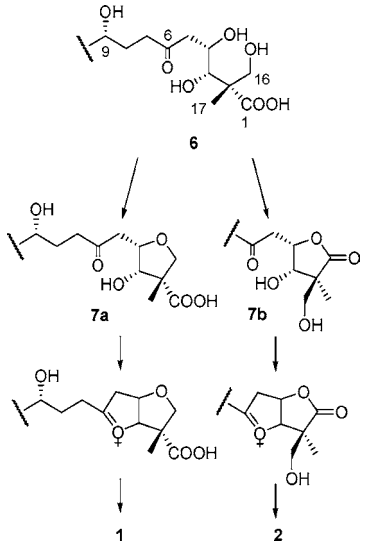
once for the less complex fungal substances cephalosporolides E and F (**3**, **4**). Ratcliffe and co-workers proposed these compounds to arise from the related lactone cephalosporolide C via a rearrangement process and suspected them to be artifacts.[7] In a recent publication, Oltra and co-workers described silica gel promoted spirocyclization of the novel fungal metabolite bassianolone (**5**), resulting in a mixture of **3** and **4**.[8]

The most intriguing feature of **2** is the unusual substitution of the γ-butyrolactone with a methyl and a hydroxymethylene group, which has only been described for secondary metabolites of the leupyrrin family.[9] In the myxobacterial leupyrrins, the γ-butyrolactone moiety is formed via an unusual branch of the mevalonate pathway. A semipinacol-like rearrangement is postulated for the formation of the unusual substitution pattern.[10] In contrast to the leupyrrins, compounds **1** and **2** are polyketides.

For the biosynthesis of **1** and **2**, we propose the polyketide precursor **6**. Ring closure can result in both an ether and an ester linkage as seen in compound **1** and **2** (Scheme 1).

Whereas **3**−**5** are straight pentaketides, **1** and **2** are composed of a methylated diketide attached to a highly modified octaketide via an ester link. The significant difference between the ascospiroketals (**1**, **2**) and the cephalosporolides E and F (**3**, **4**) is the presence of two additional

Scheme 1. Proposed Biosynthesis of **1** and **2**



carbon atoms attached to C-2 of the octaketide part of the former. These atoms (C-16 and C-17) make the herein described structural types unique and raise intriguing biosynthetic questions. As extender units other than malonyl-CoA are not known from fungal polyketides, these two carbons most probably arise from geminal biomethylation via SAM. Geminal biomethylation is a very rare process and has to our knowledge not been observed in fungal polyketides. Among bacterial polyketides, yersiniabactin is known as the only proven example for geminal biomethylation via SAM.[11,12] We have examined the biosynthesis of **1** and **2** by feeding $^{13}C_2$ acetate. The compounds were produced in extremely small amounts. Though not all homonuclear couplings could be analyzed, our data indicated that the carbon skeleton of ascospiroketals is the result of an ester linkage between a methylated diketide and a modified octaketide.

Acknowledgment. This project is part of the Graduate College GRK677 and is financially supported by the Deutsche Forschungsgemeinschaft (DFG).

Supporting Information Available: Experimental details and one- and two-dimensional NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0626802

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