ORGANIC LETTERS

2013
Vol. 15, No. 17
4320–4323

Structures of Spiroindicumides A and B, Unprecedented Carbon Skeletal Spirolactones, and Determination of the Absolute Configuration by Vibrational Circular Dichroism Exciton Approach

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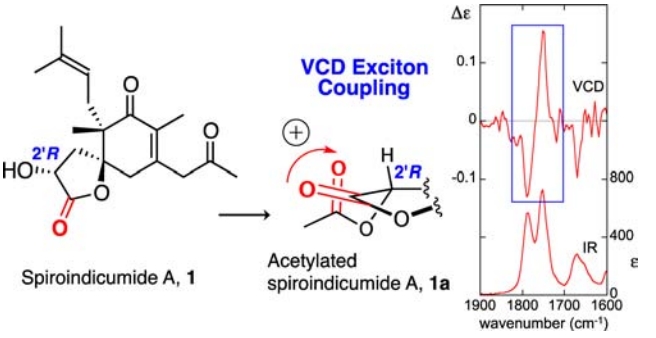
Received June 20, 2013

ABSTRACT



Spiroindicumides A (1) and B (2), novel spirolactone polyketides, were isolated from a filamentous fungus, *Chaetomium indicum*, cultivated in the presence of a histone deacetylase inhibitor. Their structures including relative configurations were determined by spectroscopic analyses. Their absolute configurations were unambiguously assigned by the vibrational circular dichroism (VCD) exciton chirality method using only ca. 0.3 mg of each sample. This study presents the first application of the VCD exciton approach to novel natural products. A possible biosynthetic pathway of the new compounds was also proposed.

Natural products have historically contributed to the development of clinically relevant drugs. However, research into natural products has declined in the pharmaceutical industry due to the decrease in the chances of isolating promising drug leads with unprecedented molecular frameworks and the slow process of identifying such novel stereostructures.[1] Solutions to these two problems would facilitate drug discovery through natural products.

The potential of filamentous fungi as a rich source of natural products has been unveiled by recent genome sequencing studies that have shown the presence of many cryptic biosynthetic gene clusters encoding uncharacterized secondary metabolites.[2] Such novel compounds have been isolated with difficulty because the gene expressions are transcriptionally suppressed under standard culture conditions.[3] Access to secondary metabolites hidden in the

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10.1021/ol401741z
Published on Web 08/23/2013

cryptic biosynthetic pathways would increase the opportunity to discover new drug candidates. Recently, the use of chemical epigenetic modifiers, such as histone deacetylase (HDAC) and/or DNA methyltransferase inhibitors, in fungal cultivation has been recognized as a promising approach to obtaining the cryptic secondary metabolites.[4] Indeed, this chemical epigenetic method developed by us has enabled easy access to various novel skeletal natural products.[5]

Structural elucidation processes remain a bottleneck in studies on a series of compounds with an uncommon structural motif and/or available in limited amount. Although there is still no common procedure to determine the absolute configurations of such compounds, the recently developed vibrational circular dichroism (VCD) technique in conjunction with density functional theory (DFT) calculations has been widely used in absolute structure elucidations.[6] Nevertheless, the low sensitivity of the vibrational absorption transitions (1−10 mg is usually needed to obtain a VCD spectrum) and heavy computational demands have hampered the utility of this approach. Most recently, a VCD exciton coupling approach for assignment of absolute configuration was shown to be a sensitive and convenient method that can overcome the limitations of conventional VCD methods.[7] Analogous to the ECD exciton chirality method developed by Harada and Nakanishi,[8] this technique, tentatively called the VCD exciton chirality method, relies on a bisignate VCD couplet having a sign that reflects the relative spatial orientations of two IR chromophores. Namely, the positive dihedral angle of two chromophores, such as carbonyl groups, generates a positive−negative couplet from lower to higher frequencies, and the negative angle gives a negative−positive couplet. Moreover, the ability to amplify the intensity of a VCD signal should enable assignment of absolute stereochemistry using less than a milligram of the sample. The approach has been tested for a small number of compounds with known absolute stereochemistries,[7] and its use in the characterization of new natural products is yet to be studied.

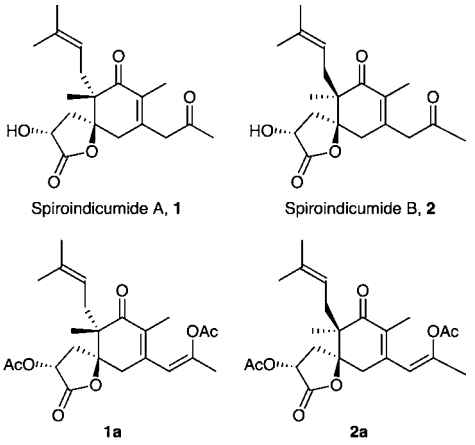

Figure 1. Structures of **1** and **2** and their diacetylated derivatives, **1a** and **2a**.

In our ongoing efforts in the search for novel skeletal secondary metabolites using chemical epigenetic methods, we found that the cultivation of *Chaetomium indicum* in the presence of 500 μM suberoyl bis-hydroxamic acid (SBHA) (a HDAC inhibitor) showed significantly enhanced its secondary metabolite production, leading to the isolation of aromatic polyketides with novel skeletons as major constituents.[5d] Scaled-up cultivation under the same condition yielded the spiroindicumides A, **1** (3.9 mg), and B, **2** (2.8 mg), as minor constituents bearing an unprecedented spirolactone core. Herein, we discuss their structure elucidation and demonstrate the use of the VCD exciton chirality method in the determination of their absolute configurations in a submilligram scale. This study presents the first application of the VCD exciton approach to novel natural products.

The molecular formula of spiroindicumide A, **1**, $C_{19}H_{26}O_5$, was deduced from its HREIMS at m/z 334.1774 $[M]^+$ (calcd 334.1780). The ^{13}C NMR and IR spectra were used to assign the three carbonyl functions to a γ-lactone (δ_C 176.3, 1777 cm^{-1}), an enone (δ_C 199.1, 1672 cm^{-1}), and a saturated ketone (δ_C 204.0, 1716 cm^{-1}). In addition to these carbons, the ^{13}C NMR spectrum displayed signals due to three sp^2 quaternary, one sp^2 tertiary, one oxygenated quaternary, one oxymethine, one quaternary, four methylene, and five methyl carbons (see SI, Table S1). The presence of 2-oxopropyl [δ_C 30.3 (C-1), 204.0 (C-2), 48.8 (C-3); δ_H 2.23 (H_3-1), 3.58 (d, J = 16.9 Hz, Ha-3), 3.26 (d, J = 16.9 Hz, Hb-3)], 3,3-dimethylallyl [δ_C 135.3 (C-14), 117.6 (C-13), 33.2 (C-12), 25.9 (C-15), 17.7 (C-16); δ_H 4.96 (brt, J = 7.5 Hz, H-13), 2.42 (dd, J = 14.3, 7.5 Hz, Ha-12), 2.19 (dd, J = 14.3, 7.5 Hz, Hb-12), 1.68 (brs, H_3-15), 1.55 (brs, H_3-16)], and 2-hydroxy propionate [δ_C 176.3 (C-1′), 68.1 (C-2′), 37.4 (C-3′); δ_H 4.51 (dd, J = 9.5, 7.4 Hz, H-2′), 2.83 (dd, J = 14.3, 9.5 Hz, Ha-3′), 1.93 (dd, J = 14.3, 7.4 Hz, Hb-3′)] moieties were unambiguously verified by the HMBC and $^1H-^1H$ COSY data presented in Figure 2. The H−C long-range correlations of H_3-10/C-4, C-8, C-9; H_2-3/C-9, C-5; H_2-5/C-4, C-6,

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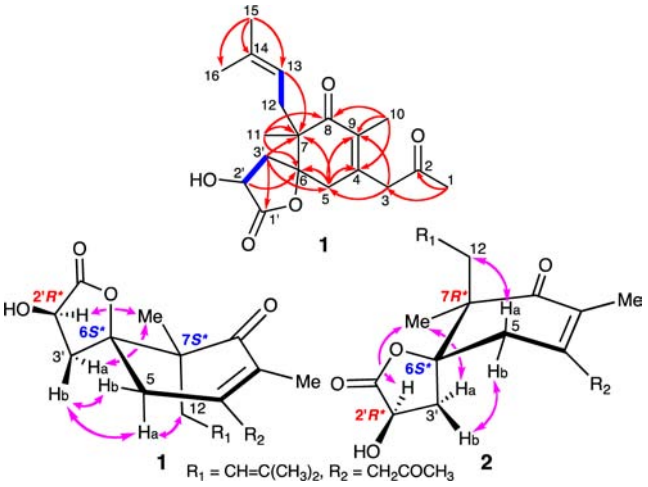

Figure 2. Key HMBC (red arrow) and ^{1}H−^{1}H COSY (blue bold line) correlations of **1** and relative configurations of **1** and **2** by 1D NOE (purple arrow) experiments.

C-7, C-9; H$_2$-3′/C-5, C-6, C-7; H$_3$-11/C-6, C-7, C-8; and H-13/C-7 indicated the presence of a cyclohexenone ring (C-4−C-9) with the substituents at C-4, C-6, C-7, and C-9 (Figure 2). Finally, the γ-lactone involving a C-1′−C-6 ester bond was identified from the molecular formula of **1**, thereby elucidating the unprecedented spirolactone planar structure of **1**. The relative configuration of **1** was determined on the basis of 1D NOE analyses (Figure 2). The NOEs of Ha-5/Ha-12 and Hb-3′/Ha-5, Hb-5 assigned the 3,3-dimethylallyl and C-3′ methylene moieties on the cyclohexenone ring to be pseudo-axial and pseudo-equatorial, respectively. The NOE correlation between H$_3$-11 and H-2′ indicated their spatial relationship, shown in Figure 2. The relative configuration was thus characterized as 2′*R**,6*S**,7*S**.

Spiroindicumide B, **2**, was found to have the same planar structure as that of **1** based on its molecular formula and the physicochemical data from EIMS, IR, UV, and 1D and 2D NMR studies (Figure 2; see SI, Table S1). Compound **2** differed from **1** only in that the C-3′ methylene moiety in **2** adopted a *pseudo*-axial orientation, indicating that the configuration of **2** was 2′*R**,6*S**,7*R** (Figure 2).

We attempted to determine the absolute stereochemistries at C-2′ in both **1** and **2** by applying the advanced Mosher's method to their secondary hydroxyl groups; however, the stereochemistries of both compounds could not be deduced from the $\Delta\delta$ values (see SI, Figure S1). Considering the limited quantity of the sample available and the difficulties associated with other methods to assign the chirality, we considered the use of the VCD exciton coupling method to analyze **1** and **2**. A milligram of each of **1** and **2** was acetylated under standard conditions to yield the corresponding diacetate derivatives **1a** and **2a** (Figure 1). The VCD and IR spectra of the underivatized spiroindicumides and their derivatives were examined in $CDCl_3$ (Figure 3). The spectra of **1** and **2** at a concentration of 0.05 M showed relatively small signals with no significant peaks

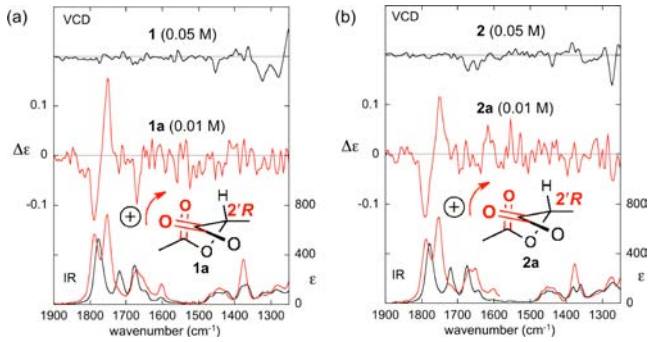

Figure 3. Comparison of VCD spectra of underivatized (black) and acetylated spiroindicumides (red) (a) **1** and (b) **2**. VCD (top) and IR (bottom) spectra and arrangement of two carbonyl groups are shown. VCD and IR spectra were collected for 90 and 2 min, respectively. Each spectrum was measured using $CDCl_3$ (l = 100 μm) and corrected by a solvent spectrum obtained under identical measurement conditions. Schematic structures are shown in their favored conformation having the *s-trans* conformation of the ester group and the *syn* relationship between the ester carbonyl and the methine hydrogen. IR spectrum of **2a** around 1550 cm^{-1} is omitted due to an apparent interference from atmospheric water IR absorption.

above 1700 cm^{-1}. By contrast, a strong bisignate VCD signal was observed in the C=O stretching region for the diacetates **1a** and **2a**, even at a concentration of 0.01 M, at which only ca. 0.3 mg of each sample was present. The results demonstrated the effectiveness of the VCD exciton approach as a signal intensifier and suggested that the use of a smaller amount of samples is possible. Moreover, both **1a** and **2a** displayed a positive−negative couplet (from lower to higher frequencies) that suggested a clockwise orientation between the two adjacent carbonyl groups at C-1′ and AcO-2′, the interactions of which would be expected to dominate the observed couplet (see SI, Figure S2). Therefore, we concluded that the absolute configuration of the C-2′ chiral center was *R* (Figure 3) and the entire absolute stereostructures were (2′*R*,6*S*,7*S*)-**1** and (2′*R*,6*S*,7*R*)-**2**.

The VCD exciton chirality method does not require theoretical calculations, and therefore, it can conveniently assign the absolute configurations of large molecules with high sensitivity. Yet, we carried out the VCD calculation of **1** and **2** to corroborate the assignments, as they are within the range of the applicability of computation. A preliminary MMFF conformational search was conducted for (2′*R*,6*S*,7*S*)-**1** and (2′*R*,6*S*,7*R*)-**2**, yielding a variety of conformers that differed in the puckering of the cyclohexenone[9a] and γ-lactone rings[7,9b] and in the orientations of the 2-oxopropyl, 3,3-dimethylallyl, and C-2′-hydroxyl side chains. Next, 20 stable structures of (2′*R*,6*S*,7*S*)-**1** and 27 stable structures of (2′*R*,6*S*,7*R*)-**2** were optimized at the DFT/B3LYP/6-31G(d) level. Five conformers of (2′*R*,6*S*,7*S*)-**1**

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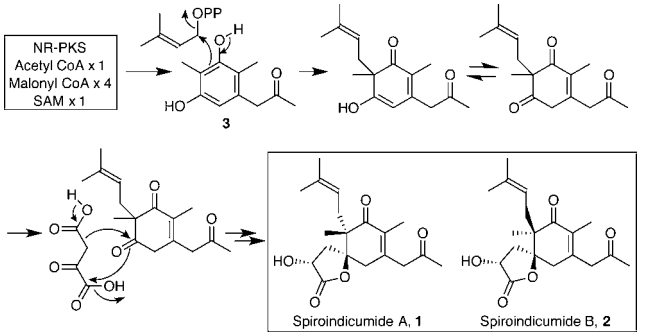
Scheme 1. Plausible Biosynthetic Pathway for **1** and **2**



and six conformers of (2′*R*,6*S*,7*R*)-**2** within 1.0 kcal/mol from the most stable respective conformers (see SI, Figure S3) were taken into account for the VCD calculations at the same level of theory, and the resultant spectra of each conformer were averaged based on the Boltzmann populations. The observed spectra for **1** and **2** agreed well with the spectra calculated for (2′*R*,6*S*,7*S*)-**1** and (2′*R*,6*S*,7*R*)-**2** (see SI, Figure S4), thereby corroborating the validity of the conclusions based on the VCD exciton coupling analysis. It should be pointed out that the computational approach for underivatized **1** and **2** was possible thanks to their high isolated amount, enough to observe meaningful VCD spectral features.

A plausible biosynthetic pathway for **1** and **2** is proposed in Scheme 1. The aromatic polyketide **3** generated *via* a nonreducing PKS (NR-PKS) pathway may be dearomatized by coupling with dimethylallyl diphosphate, followed by condensation with oxaloacetic acid to form the spirolactone core in both **1** and **2**.

In conclusion, we discovered two novel skeletal spirolactone polyketides, spiroindicumides A (**1**) and B (**2**), from *C. indicum* by using the chemical epigenetic method. Their absolute stereochemistries, which could not be determined using the advanced Mosher's method, were unambiguously and rapidly elucidated by the VCD exciton approach using the samples on the order of 0.1 mg. This study demonstrated the potential of the VCD exciton methodology for simplifying the process of characterization of stereostructures in novel structural motifs.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 25293022, 25108702, 24310151) from the Ministry of Education, Science, Sports and Culture of Japan.

Supporting Information Available. Figures, experimental methods, full spectroscopic data, and NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.