DOI: 10.1002/ejoc.200900422

Assignment of the Absolute Configuration of *Concentricolide* – Absolute Configuration Determination of Its Bioactive Analogs Using DFT Methods

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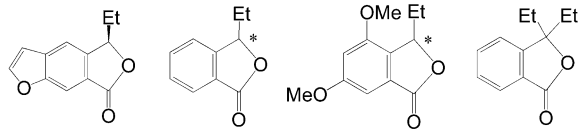
Keywords: Configuration determination / Biological activity / Density functional calculations / Chirality / Circular dichroism

The configuration of *concentricolide* was assigned as (*S*). The configuration of its three analogs, which have anti-HIV-1 activity, were predicted by optical rotation values obtained by the B3LYP/aug-cc-pVDZ//B3LYP/6-31+G(d) and B3LYP/aug-cc-pVDZ//MP2/6-311+G(d) methods. The two methods predict very close optical rotation magnitudes for all three chiral analogs of *concentricolide*. The two methods were applied in optical rotation predictions for seven other *concentricolide* analogs. Circular dichroism calculations were performed for four of the seven analogs at the B3LYP/aug-cc-pVDZ level.

Introduction

Concentricolide (**1**), obtained from Daldinia *concentrica*, exhibits anti-HIV-1 activity.[1a] Its absolute configuration remained to be confirmed as (*R*) or (*S*).[1b–1c] Its analogs **2** and **3** were synthesized by catalytic enantioselective addition of diethylzinc to the corresponding aldehydes by using our previously reported methods.[2] High enantioselectivities up to 99%*ee* were achieved for **2** and 63%*ee* for **3** was obtained. Compounds (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, (*S*)-**3**, (±)-**2**, and (±)-**3** were used for anti-HIV studies. Compound (*R*)-**3** had the best activity (EC_{50} = 28 μM) in MT4+HIV-LLAI tests. Zidovudine was used as the control (EC_{50} = 12 μM). The (*R*) configurations of **2** and **3** exhibited better anti-HIV-1 activity than the corresponding (*S*)-isomers. If there is no stereogenic center in the lactone moiety, such as in 3,3-diethylisobenzofuran-1(3*H*)-one (**4**), very low activity against HIV-1 was observed. This study reports the absolute configuration reassignment for **1** and absolute configuration determinations for **2** and **3** by using the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) and B3LYP/aug-cc-pVDZ//MP2/6-31+G(d) methods, respectively. The absolute configurations of seven other compounds that have chiral lactone moieties, essential in anti-HIV-1 activity, were investigated by using the above two methods. These configurations will be useful for synthetic studies.



Concentricolide (**1**) **2** **3** **4**

Density functional theory (DFT) methods that are used to compute optical rotations for chiral compounds have matured after an early fundamental study in 1997 by Polavarapu who used Hartree–Fock (HF).[3] Stephens and coworkers reported many good calculations of optical rotation (OR), vibrational circular dichroism (VCD), and electronic circular dichroism (CD) with DFT methods to identify natural product absolute configurations.[4] Other methods, such as coupled-cluster (CC)[5]or coupled-cluster single doublet (CCSD) methods[6] have been used for absolute configuration determinations in the research groups of Crawford and Ruud. In the past decade, Wiberg,[7] Wipf, Kondru, Beratan, Ribe, and Goldsmith,[8,9] Grimme and Ahlrichs,[10] Pedersen,[11] Giorgio,[12] Nafie,[13] Vaccaro,[14] and others[15] explored several computationally effective approaches for OR calculations that were used in stereogenic center determinations. Recently, DFT methods provided benefits in the absolute configuration determination of chiral natural products.[16] In this study, we computed ORs to determine the absolute configurations for structures **1**–**3** and **7**–**13** and both the ORs and CDs for **10**–**13**.

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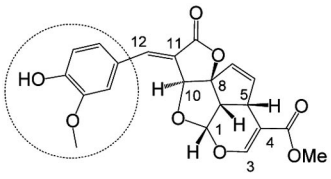
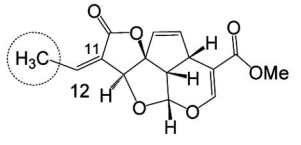
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900422.

Computational Methods

The sizes of substituents that are close to stereogenic centers often affect the sign of ORs. For example, compounds **5** and **6** have the same stereogenic centers at C-1, C-5, C-8, C-9, and C-10. The difference is that the substituent on C-12 is a methyl group in **6** and a (3-methoxy-4-hydroxy)-phenyl group in **5** (in cycles of dashes).[17a,17b] Stephens and co-workers predicted that they had opposite OR signs by using the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) method.[17c] The phenyl ring had a large effect on its OR.



(1*R*,5*S*,8*S*,9*S*,10*S*)-**5**, *oruwacin*
Exp.: $[\alpha]_D$ = +193
Calcd.: $[\alpha]_D$ = –193



(1*R*,5*S*,8*S*,9*S*,10*S*)-**6**, *plumericin*
Exp.: $[\alpha]_D$ = +198
Calcd.: $[\alpha]_D$ = +195

Compounds **1** to **3** are not as complex as **5** or **6**. However, there are two flexible –OMe groups on the aromatic ring in **3**. This may have a big effect on the OR values of **3**. Thus, the best way to predict absolute configuration of **1**–**3** is to compute their ORs.

Three stable geometries were found for compounds **1** and **2**, respectively. Compound **3** has two flexible –OMe groups. Thus, a conformational search for **3** was performed by using AM1 force field with the HyperChem package. A total of 83 AM1-optimized geometries with low energy (from 0 to 5 kcal mol^{-1}) were found in the global energy minimum and selected for further optimizations at the B3LYP/3-21G* level. A total of 33 low-energy geometries (energies from 0 to 3 kcal mol^{-1}) were selected for further optimization at the B3LYP/6-31G(d) level. Finally, 21 geometries, which had relative energies in the 0–2.0 kcal mol^{-1} range were used in OR calculations at the B3LYP/aug-cc-pVDZ level. This is method A mentioned below. To investigate the effect of the computational methods used in geometry optimizations on the OR values, all 21 B3LYP/6-31G(d)-optimized geometries were further re-optimized at the MP2/6-311+G(d) level, and these 21 MP2/6-311+G(d)-optimized geometries were used in OR computations again at the B3LYP/aug-cc-pVDZ level. This procedure is method B.

Results and Discussion

The calculated OR values of compounds **1** to **3** are listed in Table 1. The OR values of **1** were [*α*] = –43.8 for (*S*)-**1** by using method A and [*α*] = –66.1 for method B. Energetics of the B3LYP/6-31G(d)-optimized geometries and the MP2/6-311+G(d)-optimized geometries were recomputed at the B3LYP/aug-cc-pVDZ level in methanol by using the PCM model. These energy magnitudes were then used in the OR computations in methanol for (*S*)-**1** again. The OR values in methanol were [*α*] = –43.4 by using the B3LYP/6-31G(d)-optimized geometries or [*α*] = –64.5 by using the MP2/6-311+G(d)-optimized geometries, respectively. This exhibited that the ORs obtained in the gas phase for lactone **1** were almost the same as those obtained in methanol. Thus, the ORs computations in methanol for other lactones were not performed. Because **1** has only one stereogenic center, (*R*)-**1** must have a positive OR value. Experimentally, the recorded OR value for **1** was [*α*] = –59.2. The experimental OR value and sign are close to those predicted for (*S*)-**1** rather than (*R*)-**1**. Thus, the absolute configuration for *concentricolide* (**1**) should be (*S*).

The OR values for **2** were measured by using its pure enantiomer (>99%*ee*) obtained by the enantioselective additions of diethylzinc to aldehyde ester catalyzed by chiral ligands that we previously reported.[2a] The predicted OR sign agreed well with the reported results.[18] The OR magnitudes predicted by using the two methods ([*α*] ≈ –100) were close to our experimental OR value ([*α*] = –81.9). The OR values of **3** were measured twice. The *ee*% of **3** was 63% where **3** was synthesized by the catalytic enantioselective addition, and the experimental OR was [*α*] = –61.2 in methanol. After the enantiomer mixtures were separated on a Chiralcel-OD column, one enantiomer was obtained in over 99%*ee*. The OR of the major enantiomer was [*α*] = –98.8 in methanol.[19] The predicted OR values were [*α*] = –102.8 (method A) and [*α*] = –83.2 (method B). These predicted OR values are in excellent agreement with the experimental values. The OR ([*α*] = –102.8) predicted by using method A is almost the same as the magnitude of the experimental OR ([*α*] = –98.8).

Experimental OR values for **1**, **2**, and **3** are very close to the predicted OR values (Table 1, Entries 2, 3, and 5), respectively. These results show that methods A and B used in the OR calculations may be applied for configuration predictions of other similar chiral compounds.

Table 1. The computed and experimental OR values for compounds **1** to **3** in the gas phase.

Entry	Compound	Purity [%]	Calcd. $[\alpha]_D$	Computational method	Configuration	Exp. $[\alpha]_D$[a]
1	**1**	97	–43.8	A	*S*	–59.2
2			–66.1	B		
3	**2**	99	–97.3	A	*S*	–81.9, –76.0[18]
4			–102.0	B		
5	**3**	99[b]	–102.8	A	*S*	–98.8
6			–83.2	B		

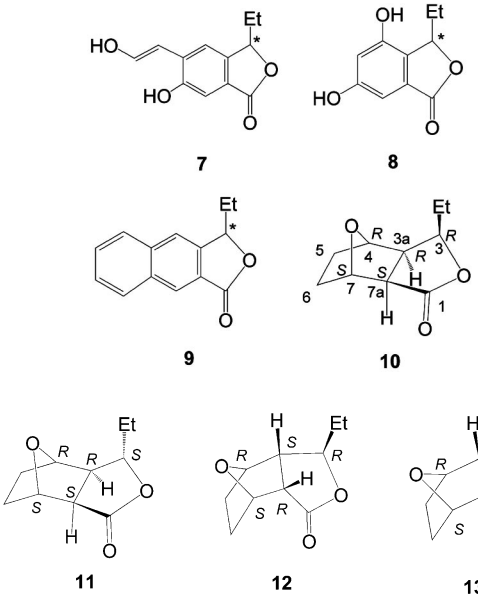
[a] The enantiomers were resolved by using a Chiralcel-OD-H column and over 99%*ee* was recorded. [b] The Chiralcel OD-H column was used to isolate the enantiomers of **3** by HPLC. The pure enantiomer was used for OR determination and anti-HIV-1 activity studies.

Table 2. Computed optical rotations and absolute configurations for compounds **7**–**13**.

Entry	Compound	Calcd. $[\alpha]_D$	Numbers of stable geometry used[a]	Computational method	Absolute configuration
1	**7**	–143.3	10	A	*S*
2	**8**	–83.4	3	A	*S*
3		–94.7	3	B	
4	**9**	–157.1	3	A	*S*
5	**10**	+121.4	3	A	(3*R*,3*aR*,4*R*,7*S*,7*aS*)
6		+136.9	3	B	
7	**11**	+34.5	3	A	(3*S*,3*aR*,4*R*,7*S*,7*aS*)
8		+43.1	3	B	
9	**12**	+18.7	3	A	(3*R*,3*aS*,4*R*,7*S*,7*aR*)
10		+14.6	3	B	
11	**13**	–52.3	2	A	(3*S*,3*aS*,4*R*,7*S*,7*aR*)
12		–57.8	2	B	

[a] The number of stable geometries found were more than those listed in the Table. However, only the geometries that have low energies were selected for use in the OR computations. Methods A and B were discussed in the Computational Methods portion of the text.

For application in future synthetic studies, the OR values of compounds **7**–**13** were calculated. These compounds have the essential chiral lactone moiety involved in anti-HIV-1 activity. Methods A [B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d)] and/or B [B3LYP/aug-cc-pVDZ//MP2/6-311+G(d)] were both used in the OR computations. The predicted ORs, their corresponding absolute configurations, and the stable geometries used in these OR computations are listed in Table 2. Both methods predicted very similar OR values for this series of chiral compounds. After the syntheses of these compounds by asymmetric methods and obtaining their OR values, it will now be possible to identify their absolute configurations.



The computed OR for **11** is $[\alpha] \approx +30$ to +50, and that of **12** is $[\alpha] \approx +10$ to +20. If the OR values for **11** or **12** were eventually found experimentally to be between $[\alpha] = +20$ and +30, it would be difficult to assign their absolute configurations on the basis of only their OR values. Thus, circular dichroism computations for **10** to **13** were performed at the B3LYP/aug-cc-pVDZ level. Figure 1 illustrates these computed plots. Fortunately, the CD spectra for compounds **11** and **12** have big differences. Thus, even if the recorded OR magnitudes for **11** or **12** are between $[\alpha] = +20$ and +30, their absolute configurations could be determined by comparing the predicted CD spectra to each other. Moreover, the NMR spectra of **11** and **12** will be markedly different, and so, a combination of 1D and 2D experiments could also distinguish **11** from **12**.

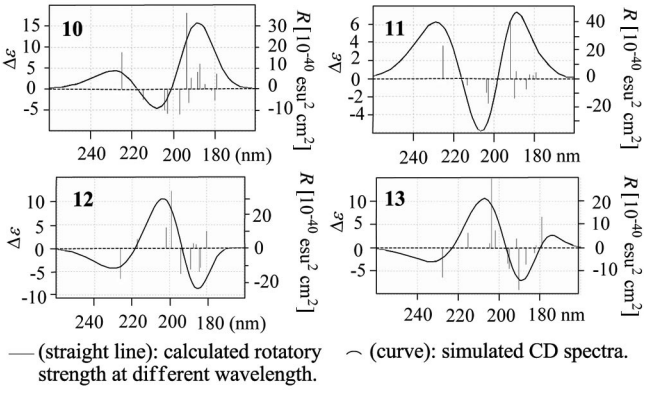


Figure 1. The calculated CD spectra for compounds **10**–**13**.

Conclusion

In this study, the absolute configuration of *concentricolide* was assigned as (*S*) by using DFT methods. Both B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) and B3LYP/aug-cc-pVDZ//MP2/6-31+G(d) computations gave good OR predictions. Analogs of *concentricolide* with anti-HIV bioactivity were synthesized and their absolute configurations were predicted by using the same methods. The absolute configurations of other chiral compounds that may have potent anti-HIV bioactivity were predicted by using the same methods as well as the calculated CD spectra. The predictions for the absolute configurations of these compounds will be helpful for further synthetic study of these compounds. B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) computations are economical and recommended for similar compound OR calculations. Further synthetic studies

aimed at the application and testing of these predictions and bioactivities are underway in our group.

Experimental Section

The OR values for all compounds were recorded at 589 nm in methanol at room temperature.

Catalytic Addition of Diethylzinc to Methyl-2-formylbenzoate and Methyl 2-Formyl-4,6-dimethoxybenzoate: A Et_2Zn solution (1 M in *n*-hexane, 0.77 mL) was added by syringe to a suspension of LiCl (0.38 mM) in dry toluene (8 mL) under an atmosphere of N_2 at room temperature. Then, the chiral ligand, (*S*)-2-[(3,3-dimethylbutyl)(methyl)amino]-3-ethyl-1-(1*H*-indol-3-yl)pentan-3-ol or 3-[(1*S*,3*S*)-2-methyl-1-neopentyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]-indol-3-yl]pentan-3-ol[1a,1b] (0.018 mM), in toluene (3 mL) and methyl-2-formylbenzoate (0.36 mM) or methyl 2-formyl-4,6-dimethoxybenzoate was added by syringe at –15 °C. The reaction solution then was stirred for 10 h at –15 °C. After quenching with 5% HCl aqueous solution, the mixture was extracted with ethyl acetate (3 × 30 mL). The combined extract solution was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 10% ethyl acetate in petroleum ether) to give the product mixture. Yields were calculated after isolation by preparative high-performance liquid chromatography (PHPLC) on a YMC-Pack SIL column (*i*PrOH/petroleum ether, 2:98; flow rate = 6 mL min^{-1}; UV detector at 278.5 nm). See Supporting Information for more experimental details.

3-Ethyl Phthalide (2): Yield: 69%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.87 (d, J = 7.6 Hz,1 H), 7.70 (dd, J = 7.1, 7.6 Hz, 1 H), 7.53 (m, 1 H), 7.51 (m, 1 H), 5.48 (dd, J = 4.3, 7.0 Hz, 1 H), 2.14 (m, 1 H), 1.83 (m, 1 H), 0.99 (t, J = 7.3 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.2, 149.3, 133.6, 128.6, 125.7, 124.9, 121.5, 81.9, 27.1, 8.3 ppm. MS (EI): m/z = 162 [M^+].

3-Ethyl-4,6-dimethoxyphthalide (3): Yield: 41%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.90 (s, 1 H), 6.66 (s, 1 H), 5.43 (dd, J = 3.1, 7.1 Hz, 1 H), 3.85 (s, 1 H), 2.26 (m, 1 H), 1.77 (m, 1 H), 0.90 (t, J = 7.3 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.8, 162.3, 154.9, 130.8, 128.7, 104.7, 98.4, 81.5, 55.8, 55.5, 25.8, 8.6 ppm. MS (EI): m/z = 222 [M^+], 193, 133, 77. HRMS (EI): calcd. for $C_{12}H_{14}O_4$ [M^+] 222.0892; found 222.0915.

3,3-Diethylisobenzofuran-1(3*H*)-one (4): Yield: 90%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.79 (d, J = 7.7 Hz, 1 H), 7.61 (m, 1 H), 7.44 (m, 1 H), 7.30 (d, J = 7.5 Hz, 1 H), 2.03 (m, 2 H), 1.87 (m, 2 H), 0.63 (t, J = 7.5 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 151.6, 133.8, 128.6, 126.8, 125.1, 120.9, 90.6, 31.0, 7.2 ppm. MS (EI): m/z = 190 [M^+].

Bioactivity Tests: cytotoxicity was measured by the MTT method.[20] The concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined. The inhibition rate and EC_{50} based on the p24 antigen expression level were calculated.[21] The selectivity index (SI) was calculated as CC_{50}/EC_{50}. The compound with the highest anti-HIV-1 activity was (*R*)-**3**. Its EC_{50} was 0.028 mM in the HIV- LAI/MT-4 cell culture assay,[22] and the SI for this was over 10.4. Zidovudine was used as the control (EC_{50} = 0.012 mM). In contrast, the EC_{50} of (*R*)-**2** was 0.147 mM, and its SI was only 1.6. Other stereogenic isomers did not exhibit good anti-HIV-1 activities. In the anti-HIV-1 study, compounds with the (*R*) configuration exhibited better activity than their (*S*) configuration enantiomers. The stereogenic center was then removed. For example, 3,3-diethylisobenzofuran-1(3*H*)-one (**4**) was synthesized. As expected, it showed a similar anti-HIV-1 activity to that of (*R*)-**2**. See Supporting Information for more test details.

Supporting Information (see footnote on the first page of this article): Experimental results, ^{1}H and ^{13}C NMR spectra, OR computational results, CD results, and details of the anti-HIV-1 bioactivity tests.

Acknowledgments

H.-J. Z. gratefully acknowledges financial support from the National Natural Science Foundation of China (30770235 and 30873141), Chinese Academic of Sciences (CAS) (YZ-06-01), 973 Program (2009CB522304), Key State Laboratory of Virology of Wuhan (2007014), and Key State Laboratory of Phytochemistry and Plant Resources in West China of Kunming Institute of Botany. The Super-Computational-Center of Yunnan University is thanked for computational support.

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Received: April 17, 2009
Published Online: July 3, 2009

