Absolute Configuration of Actinophyllic Acid As Determined through Chiroptical Data[⊥]

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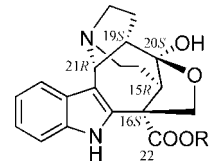
The absolute configuration of actinophyllic acid (−)-**1**, an alkaloid with an unprecedented 2,3,6,7,9,13c-hexahydro-1*H*-1,7,8-(methanetriyloxymethano)pyrrolo[1′,2′:1,2]azacino[4,3-*b*]indole-8(5*H*)-carboxylic acid skeleton isolated from *Alstonia actinophylla*, was determined through the study of its corresponding methyl ester **2**. Racemic **2** was separated into (+)-**2** and (−)-**2** enantiomers, and they were assigned unambiguously as 15*S*,16*R*,19*R*,20*R*,21*S* and 15*R*,16*S*,19*S*,20*S*,21*R*, respectively, by the use of optical rotation and electronic circular dichroism. Finally, (−)-**2** was characterized as the methyl ester of naturally occurring (−)-**1**. The assigned 15*R*,16*S*,19*S*,20*S*,21*R*-configuration of (−)-**1** is consistent with a proposed biosynthetic pathway.

The alkaloid actinophyllic acid [(−)-**1**] was isolated from the water and methanol extracts of the tree *Alstonia actinophylla*, collected on the Cape York Peninsula, Queensland, Australia, as a carboxypeptidase U inhibitor.[1] Actinophyllic acid has an unprecedented 2,3,6,7,9,13c-hexahydro-1*H*-1,7,8-(methanetriyloxymethano)pyrrolo[1′,2′:1,2]azacino[4,3-*b*]indole-8(5*H*)-carboxylic acid skeleton, as confirmed by the total synthesis of (±)-**1**.[2] Although a biogenetic pathway starting from tryptamine and (−)-secologanin glucoside has been proposed to rationalize this unique structure,[1] there has been no experimental evidence on the absolute configuration of (−)-**1**. Elucidation of its absolute stereochemistry is beneficial to obtain insight into its biosynthesis and biological activity.

Theoretical calculations using density functional theory (DFT) of chiroptical properties such as optical rotation,[3−6] electronic circular dichroism (ECD),[7−10] and vibrational circular dichroism (VCD)[11−14] have been established as a reliable and convenient method for determination of stereochemistry of chiral molecules. Each chiroptical method has its own advantages and disadvantages in terms of sensitivity, reliability, and restriction of measurement conditions, and therefore some are preferable to others for a particular molecule of interest. Recent reports have found that the use of more than one chiroptical technique will lead to unambiguous assignments.[15−17] In continuation of our studies on chiral natural products,[18,19] we have conducted a spectroscopic study on actinophyllic acid. The optical properties of the actinophyllic acid **1** were expected to be difficult, since intermolecular interaction with solute and solvent molecules may change spectroscopic properties from those simulated for the single molecular state.[5,20−22] Therefore we applied optical rotation and ECD to actinophyllic acid methyl ester **2**, made by the procedure previously reported.[2] Herein, we report the separation of the enantiomers of (±)-**2**, assignment of (+)-**2** as 15*S*,16*R*,19*R*,20*R*,21*S* and (−)-**2** as 15*R*,16*S*,19*S*,20*S*,21*R*, and characterization of (−)-**2** as the methyl ester of the naturally occurring enantiomer of actinophyllic acid (−)-**1** by chemical correlation.



1: R = H
2: R = Me

Results and Discussion

In order to correlate the absolute configuration of **2** with that of **1**, it was planned to conduct enantioseparation of (±)-**2** and subsequent hydrolysis, rather than direct enantioseparation of (±)-**1** followed by methylation; this approach was followed because we anticipated difficulties in separation of the acid (±)-**1** due to its higher polarity and aggregation propensity. Enantioseparation of **2** was achieved on normal-phase chiral HPLC using a CHIRALPAK AD column (1 cm Φ × 25 cm) with hexane−EtOH (70:30). The first enantiomer appeared at 10.3 min, and the second was eluted at 12.4 min (separation factor $\alpha = 1.3$, using $t_0 = 3.4$ min) (Figure 1). The first- and second-eluted enantiomers were identified to have positive and negative optical rotation, respectively (Table 1). Analytical HPLC showed that (+)-**2** was obtained as >99% ee, while (−)-**2** was found to be 90% ee, and therefore optical rotations and ECD data of (−)-**2** were corrected to 100% ee. The enantiomer (−)-**2** was characterized as the methyl ester of the naturally occurring enantiomer of actinophyllic acid (−)-**1** by chemical correlation (vide infra).

Prior to the calculation of chiroptical properties, a conformational analysis was carried out to define stable conformations and their relative energies. The initial structure for the calculation was arbitrarily built as (15*R*,16*S*,19*S*,20*S*,21*R*)-**2**, the enantiomer deduced by the proposed biosynthetic pathway starting with tryptamine and (−)-secologanin glucoside.[1] Due to the rigidity of its skeleton, an MMFF94 Monte Carlo search yielded only two conformers that differ in the orientation of the C-22 ester group within a 10 kcal/mol window. Optimization of these conformers at the DFT/B3LYP/6-31G(d,p) level without considering solvent effects did not change these geometries significantly. Their stability was verified by calculations of their vibrational frequencies. The conformers are shown in Figure 2, and their relative energies and Boltzmann populations are listed in Table 1. The conformer **2a** is 1.4 kcal/mol more stable than **2b**, thus existing as the dominant conformer with the population more than 90%. Still, the Boltzmann population of conformer **2b** is not negligible, and therefore the optical properties were calculated for the both conformers and then they are averaged according to their Boltzmann populations.

Our first plan was to conduct VCD studies in addition to those by optical rotation and ECD spectroscopy, since the VCD technique

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Table 1. Relative Energies, Boltzmann Populations, and Calculated $[\alpha]_D$ of Stable Conformers of (15*R*,16*S*,19*S*,20*S*,21*R*)-**2** and Observed $[\alpha]_D$ of **2**

	ΔE (kcal/mol)[a]	Boltzmann population[a]	calcd $[\alpha]_D$ (6-31G(d,p))[b]	calcd $[\alpha]_D$ (aug-cc-pVDZ)[b]
2a	0.000	91.4%	−111.46	−142.16
2b	1.404	8.6%	−111.81	−124.22
	Boltzmann averaged $[\alpha]_D$		−111.49	−140.63

	observed $[\alpha]_D$ (c = 0.1 [g/(100 mL)])	
	first eluted enantiomer (unnatural)	second eluted enantiomer (naturally occurring)
$CHCl_3$	+100.1	−100.3
MeCN	+102.5	N.D.[c]
MeOH	+142.9	−136.1

[a] Calculated at DFT/B3LYP/6-31G(d,p). [b] Calculated using TDDFT with B3LYP functional. [c] Not determined.

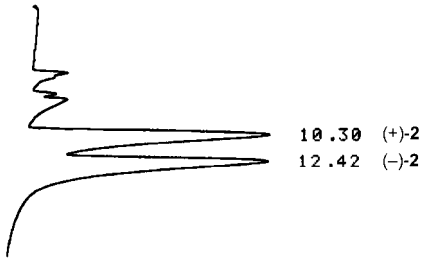

Figure 1. HPLC profile of (±)-**2** on a CHIRALPAK AD column.

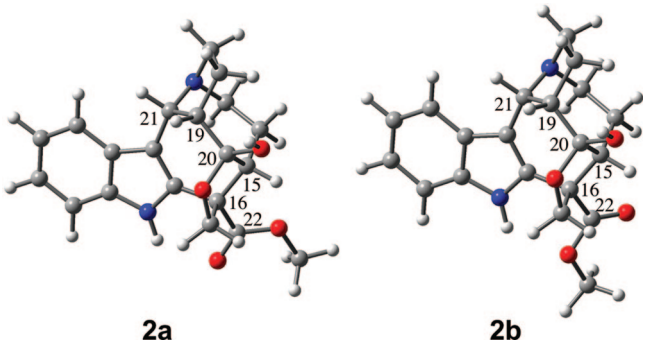

Figure 2. Two stable conformers of (15*R*,16*S*,19*S*,20*S*,21*R*)-**2** optimized at B3LYP/6-31G(d,p).

was expected to provide valuable information on its stereochemistry due to a large number of signals.[23,24] However, actinophyllic acid methyl ester **2** was found to have limited solubility for noncoordinating solvents such as CCl_4 and $CDCl_3$, which are suitable for comparison with calculated VCD spectra. For this reason, VCD measurement of **2** using $CDCl_3$ provided a spectrum with a low S/N ratio, while that using DMSO-d_6, a strongly coordinating solvent, exhibited certain discrepancies between the observed and calculated spectra, which precluded a safe assignment. In order to elucidate its absolute configuration with a limited amount of sample in hand, we decided to apply optical rotation and ECD instead of pursuing a better condition for VCD spectroscopy.

Since **2** is soluble in chloroform, acetonitrile, and methanol only at a lower concentration (c 0.1 [g/(100 mL)]), $[\alpha]_D$ values of **2** were measured in these solvents to examine the influence of solvents. Table 1 shows that optical rotations of **2** uniformly exhibit large values around 100 with slight differences depending on solvents. According to recent studies,[15,25] such large $[\alpha]_D$ values are favorable for reliable assignment based on time-dependent density functional theory (TDDFT) calculation. Optical rotations of **2a** and **2b** at 589.3 nm were calculated employing TDDFT with the B3LYP functional and the 6-31G(d,p) or aug-cc-pVDZ basis set and then averaged according to their Boltzmann population (Table 1). Both basis sets predicted similar negative values that are in good agreement with the experimental $[\alpha]_D$ of (−)-**2**, thus

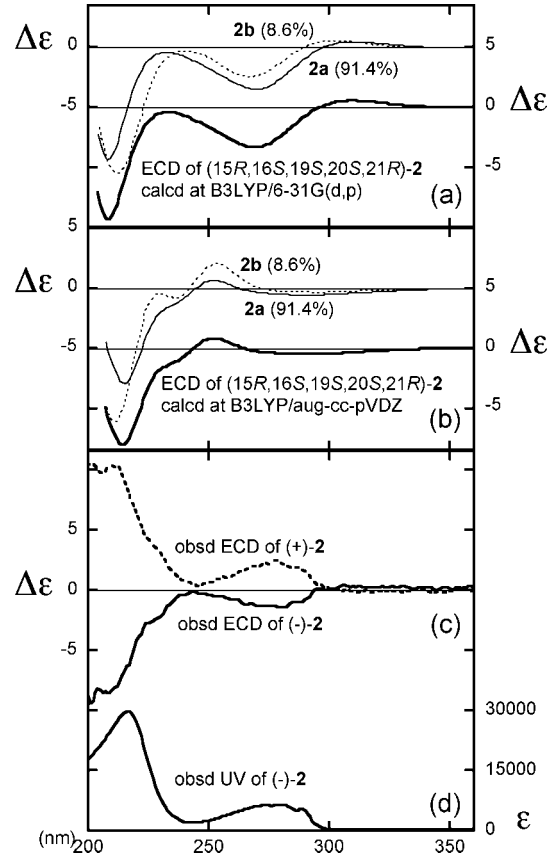

Figure 3. (a) Calculated ECD spectra of (15*R*,16*S*,19*S*,20*S*,21*R*)-**2** at B3LYP/6-31G(d,p) and (b) at B3LYP/aug-cc-pVDZ. (c) Observed ECD and (d) UV spectra of **2** in methanol (0.25 mM).

Table 2. Comparison of Observed Optical Rotations of **1** Derived from (−)-**2** with the Reported Value

	concentration [g/(100 mL)][a]	$[\alpha]_D$
1 from (−)-**2**	0.02	−41
	0.004	−29
	0.004 (+0.95 equiv NaOMe)	−65
natural **1**[b]	0.001	−29

[a] Measured in methanol. [b] Ref 1.

clearly suggesting the absolute configuration of (−)-**2** as 15*R*,16*S*,19*S*,20*S*,21*R* and (+)-**2** as 15*S*,16*R*,19*R*,20*R*,21*S*.

ECD spectroscopy was then applied to further confirm the assignment. The ECD and UV spectra of **2** were measured in methanol as shown in Figures 3c and 3d. Theoretical calculations of the ECD of (15*R*,16*S*,19*S*,20*S*,21*R*)-**2** were performed using TDDFT with the B3LYP functional and the 6-31G(d,p) or aug-cc-pVDZ basis set (Figures 3a and 3b). The theoretical spectrum by 6-31G(d,p) reproduced well the experimental spectrum for (−)-**2**: a negative broad band around 275 nm, an upward tendency close

to zero around 240 nm, and a negative peak at ~210 nm. Similarly, the spectrum calculated at aug-cc-pVDZ also showed a broad negative band at the longer wavelength region and a strong negative peak at the shorter wavelength region, as observed for (−)-**2**. Although in the calculated spectrum by aug-cc-pVDZ the upward region around 240 nm became weakly positive, this basis set reproduced the negative shoulder at ~230 nm well. These comparisons between theoretical and experimental spectra confirmed the 15*R*,16*S*,19*S*,20*S*,21*R*-configuration of (−)-**2**, consistent with the conclusions deduced from the optical rotations.

Chemical correlation between **2** and **1** was conducted by hydrolysis of its methyl ester in aqueous HCl solution according to the procedure reported in the literature.[2] The HCl salt of **1** derived from optically active (−)-**2** was dissolved in methanol, and its $[\alpha]_D$ was measured to compare with that of naturally occurring **1** (lit.[1] $[\alpha]_D$ −29 (*c* 0.001, MeOH)). The concentrations of 0.02 and 0.004 were used to check whether concentration affects its sign. At the both concentrations, it showed negative values with similar intensities (Table 2). In addition, since the NMR spectrum of **1** is known to be highly influenced by its zwitterionic state, we measured the $[\alpha]_D$ value after adding 0.95 equiv of sodium methoxide to the solution.[26] As shown in Table 2, the addition of base did not affect its sign with a higher magnitude. In these measurements, all values exhibited a negative sign. Particularly, the $[\alpha]_D$ recorded at the concentration of 0.004 without base gave the same value as that reported;[1] although this could be fortuitous, this result led us to conclude that (−)-**2** is the methyl ester of naturally occurring (−)-**1**. Thus, we have established the absolute configuration of naturally occurring **1** as 15*R*,16*S*,19*S*,20*S*,21*R*.

In conclusion, we have determined the absolute configurations at all five stereogenic centers of actinophyllic acid (**1**) through the application of two chiroptical methods to the corresponding methyl ester, **2**. The assignment by the use of optical rotation and ECD is consistent with the proposed biosynthetic pathway.[1] The stereochemical information obtained in this study should contribute to further research on biological activity of actinophyllic acid and its analogues.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter at the sodium-D line using a 1 cm optical cell. UV and ECD spectra were recorded on a JASCO J-810 spectrometer with a 1 mm quartz cell. IR and VCD spectra were obtained on a Bomem/BioTools Chiral*ir* spectrometer equipped with a second elastic modulator using a 72 μm BaF_2 cell. Data were corrected by solvent data obtained at the same experimental conditions. VCD, optical rotation, and ECD data were normalized to 100% ee.

Enantioseparation of (±)-**2** was carried out on a CHIRALPAK AD column (1 cm Φ × 25 cm) using a Shimadzu LC-6A liquid chromatograph instrument equipped with a Shimadzu SPD-6AV UV−vis spectrometric detector. HPLC purification of **1** was conducted on an Inertsil ODS-3 column (2 cm Φ × 25 cm) using a Hitachi HPLC D-7000 system equipped with an L-6250 intelligent pump and an L-7400 UV detector.

Compound for Study. Racemic actinophyllic acid methyl ester (±)-**2** was obtained by dissolving racemic actinophyllic acid methyl ester trifluoroacetate[2] in a saturated $NaHCO_3$ aqueous solution and extracting it with $CHCl_3$. After the removal of the solvent, the sample (±)-**2** was loaded on the chiral HPLC column. Hydrolysis of enantioseparated actinophyllic acid methyl ester **2** to actinophyllic acid **1** was conducted following the procedure reported previously.[2]

Theoretical Calculations. Calculations were conducted for arbitrarily chosen (15*R*,16*S*,19*S*,20*S*,21*R*)-**2**, the enantiomer expected to arise from the proposed biosynthetic pathway.[1] Preliminary conformational search using MMFF94 systematic conformational searches via a Spartan02 program led to only two stable conformers within a 10 kcal/mol window. These conformers were further optimized at the DFT/B3LYP/6-31G(d,p) level without considering solvent effects using the Gaussian03 program. Optical properties were calculated for these two optimized geometries using the Gaussian03 program, and then each data set was averaged based on the Boltzmann population. ECD spectra were simulated from the first 50 singlet→singlet electronic transitions using Gaussian band shapes with 0.30 eV standard deviation.[7,27] The highest energy transition appeared at around 197 nm, and therefore the calculated ECD spectra below 207 nm are omitted since the spectra in this region are subject to change by calculating a larger number of transitions. However, attempts to calculate a larger number of transitions were abandoned due to the excessive demand on computational time.

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References and Notes

(1) Carroll, A. R.; Hyde, E.; Smith, J.; Quinn, R. J.; Guymer, G.; Forster, P. I. *J. Am. Chem. Soc.* **2005**, *70*, 1096–1099.
(2) Martin, C. L.; Overman, L. E.; Rohde, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 7568–7569.
(3) Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Phys. Chem., A* **2001**, *105*, 5356–5371.
(4) Polavarapu, P. L. *Chirality* **2002**, *14*, 768–781.
(5) Giorgio, E.; Roje, M.; Tanaka, K.; Hamersak, Z.; Sunjik, V.; Nakanishi, K.; Rosini, C.; Berova, N. *J. Org. Chem.* **2005**, *70*, 6557–6563.
(6) Stephens, P. J.; Pan, J. J.; Devlin, F. J.; Cheeseman, J. R. *J. Nat. Prod.* **2008**, *71*, 285–288.
(7) Diederich, C.; Grimme, S. *J. Phys. Chem., A* **2003**, *107*, 2524–2539.
(8) Schühly, W.; Crochett, S. L.; Fabian, W. M. F. *Chirality* **2005**, *17*, 250–256.
(9) Hussain, H.; Krohn, K.; Floerke, U.; Schulz, B.; Draeger, S.; Pescitelli, G.; Antus, S.; Kurtán, T. *Eur. J. Org. Chem.* **2007**, *72*, 292–295.
(10) Ding, Y.; Li, X.-C.; Ferreira, D. *J. Org. Chem.* **2007**, *72*, 9010–9017.
(11) Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *Chem. Phys. Lett.* **1996**, *252*, 211–220.
(12) Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. *Chirality* **2003**, *15*, 743–758.
(13) Monde, K.; Taniguchi, T.; Miura, M.; Vairappan, C. S.; Suzuki, M. *Tetrahedron Lett.* **2006**, *47*, 4389–4392.
(14) Cerda-García-Rojas, C. M.; Catalán, C. A. N.; Muro, A. C.; Joseph-Nathan, P. *J. Nat. Prod.* **2008**, *71*, 967–971.
(15) Stephens, P. J.; McCann, D. M.; Devlin, F. J.; Smith, A. B. *J. Nat. Prod.* **2006**, *69*, 1055–1064.
(16) Polavarapu, P. L. *Chem. Rec.* **2007**, *7*, 125–136.
(17) Polavarapu, P. L. *Chirality* **2008**, *20*, 664–672.
(18) Schlingmann, G.; Taniguchi, T.; Haiyin, H.; Bigelis, R.; Yang, H. Y.; Koehn, F. E.; Carter, G. T.; Berova, N. *J. Nat. Prod.* **2007**, *70*, 1180–1187.
(19) Taniguchi, T.; Monde, K.; Nakanishi, K.; Berova, N. *Org. Biomol. Chem.* **2008**, *6*, 4399–4405.
(20) He, J.; Wang, F.; Polavarapu, P. L. *Chirality* **2005**, *17*, S1−S8.
(21) Devlin, F. J.; Stephens, P. J.; Bortolini, O. *Tetrahedron: Asymmetry* **2005**, *16*, 2653–2663.
(22) Kuppens, T.; Herreboout, W.; van der Veken, B.; Bultinck, P. *J. Phys. Chem., A* **2006**, *110*, 10191–10200.
(23) Nafie, L. A. *Nat. Prod. Commun.* **2008**, *3*, 451–466.
(24) Burgueño-Tapia, E.; Joseph-Nathan, P. *Phytochemistry* **2008**, *69*, 2251–2256.
(25) Stephens, P. J.; McCann, D. M.; Cheeseman, J. R.; Frisch, M. J. *Chirality* **2005**, *17*, S52−S64.
(26) In ref 2, ca. 1 equiv of NaDMSO-d_5 was added to a DMSO-d_6 solution of synthetic **1** to obtain the identical ^{1}H NMR spectrum reported for natural product **1**.
(27) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy−exciton coupling in organic stereochemistry*; University Science Books: Mill Valley, CA, 1983.

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