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Brasilamides A–D: Sesquiterpenoids from the Plant Endophytic Fungus *Paraconiothyrium brasiliense*

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New tricyclic sesquiterpenoids brasilamides A–D (**1**–**4**) and the known pinthunamide (**5**) have been isolated from cultures of the plant endophytic fungus *Paraconiothyrium brasiliense* Verkley. Their structures were elucidated primarily by NMR spectroscopy, and the structure of **1** was further confirmed by X-ray crystallography. The absolute configuration of the C-5 tertiary alcohol in **2** was assigned by analogy to **1** and confirmed by observing the circular dichroism of in situ generated $[Rh_2(OCOCF_3)_4]$ complex. Compounds **2**–**4** showed modest inhibitory effects on HIV-1 replication in C8166 cells. Compounds **1** and **2** possess an unprecedented 4-oxatricyclo-[3.3.1.$0^{2,7}$]nonane skeleton.

Introduction

Sesquiterpenoids incorporating the bergamotane skeleton have been reported from various natural sources. Examples of fungal metabolites include pinthunamide (**5**) isolated from *Ampulliferina* sp.,[1] ampullicin, and isoampullicin, and dihydroampullicin from *Ampulliferina*-like sp. No 27,[2,3] bergamotene from *Aspergillus fumigatus*,[4] the expansolides from *Penicillum expansum* and *Aspergillus fumigatus* Fresenius,[5,6] the massarinolins from the aquatic fungus *Massarina tunicate*,[7] and the decipienolides from a coprophilous fungus *Podospora decipiens*.[8] Whereas those of plant metabolites include tanavulgarol from *Tanacetum vulgare*[9] and the clavigerins from the liverwort *Lepidolaena clavigera*.[10,11] Plant endophytic fungi are well-known sources of bioactive natural products,[12–16] and our previous chemical studies have also afforded a variety of bioactive secondary metabolites.[17–19] During our continuous search for new bioactive compounds from this class of fungi, a strain of *Paraconiothyrium brasiliense* Verkley (M3–3341), isolated from branches of *Acer truncatum* Bunge on Dongling Mountain, Beijing, P. R. China, was grown in a solid-substrate fermentation culture. An organic solvent extract of the culture showed an inhibitory effect on HIV-1 replication in C8166 cells. Fractionation of the extract afforded four new sesquiterpenoids, which we have named brasilamides A–D (**1**–**4**), and the known compound pinthunamide (**5**; Figure 1).[1] Details of the isolation, structure elucidation, and biological activity of these compounds are reported herein.

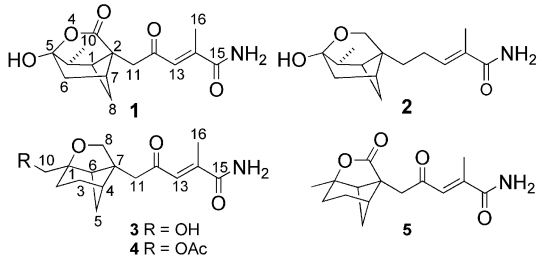

Figure 1. Metabolites **1**–**5** from *P. brasiliense*.

Results and Discussion

The molecular formula of brasilamide A (**1**) was established as $C_{15}H_{19}NO_5$ (seven degrees of unsaturation) on the basis of its HRMS (ESI) spectrum (*m*/*z* = 316.1147 [M + Na]$^+$, Δ = +0.8 mmu). Analysis of the ^{1}H, ^{13}C, and HMQC NMR spectroscopic data (Table 1) of **1** revealed three exchangeable protons, two methyl groups, three methylene units, three methines, two sp^3 quaternary carbon atoms (one oxygenated), one trisubstituted olefin, one α,β-unsaturated ketone (δ = 200.0 ppm), and two carboxy (δ = 172.6 and 170.7 ppm) carbon atoms. Interpretation of the ^{1}H–^{1}H COSY NMR spectroscopic data established an isolated proton spin system corresponding to the C-6–C-10 (through C-7, C-8, C-1, and C-9) subunit. HMBC correlations from 1-H, 6-H, and 7-H to C-2 and C-5 indicate

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that the C-5 oxygenated sp^3 quaternary carbon (δ = 103.9 ppm) atom is located between C-6 and C-9, whereas the other sp^3 quaternary carbon C-2 (δ = 45.9 ppm) atom is attached to both C-1 and C-7, completing the bicyclo-[3.1.1]heptane ring in **1**. Correlations from 11-H to C-1, C-2, C-3, C-7, and C-12 reveal the connection of C-2 to C-3 and C-11, and of the C-12 ketone carbon atom to C-11, whereas those from 13-H to C-12, C-14, C-15, and C-16 and from 16-H to C-13, C-14, and C-15 indicate that the C-13/C-14 olefin is conjugated to both the C-12 ketone and the C-15 carboxy carbon atoms. Considering the doubly oxygenated nature of C-5 (δ = 103.9 ppm) and the unsaturation requirement for **1**, the C-3 carboxy carbon atom must acylate one of the oxygen atoms attached to C-5 to form a δ-lactone moiety, thereby completing the 4-oxatricyclo-[3.3.1.$0^{2,7}$]nonane skeleton in **1**. The remaining two exchangeable protons were assigned as 15-NH_2 by default. On the basis of these data, the gross structure of **1** was established, as shown in Figure 1.

Table 1. NMR spectroscopic data for **1** in [D_6]acetone.

Pos.	δ_H [ppm] (mult., J [Hz])[a]	δ_C [ppm][b]	HMBC
1	2.20 (t, 6.0)	43.6, CH	2, 5, 7, 8, 9, 10, 11
2		45.9, C_q	
3		172.6, C_q	
5		103.9, C_q	
6	2.11 (br. d, 3.0)	41.7, CH_2	2, 5, 7, 8, 9
7	2.47 (m)	36.4, CH	1, 2, 5, 8, 11
8a	1.28 (d, 10)	36.4, CH_2	1, 2, 6, 7, 9
8b	2.67 (ddd, 10, 6.0, 5.5)		1, 6, 7, 9
9	2.25 (dd, 7.0, 6.0)	44.4, CH	1, 2, 5, 8, 10
10	0.96 (d, 7.0)	12.9, CH_3	1, 5, 9
11	3.14 (s)	44.1, CH_2	1, 2, 3, 7, 12
12		200.0, C_q	
13	6.88 (d, 1.5)	129.3, CH	12, 14, 15, 16
14		145.0, C_q	
15		170.7, C_q	
16	2.16 (d, 1.5)	14.8, CH_3	13, 14, 15
OH-5	6.40 (br. s)		
NH_2-15	6.58 (br. s); 7.16 (br. s)		

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

Ultimately, the structure of brasilamide A (**1**) was confirmed by single-crystal X-ray crystallographic analysis, and a perspective ORTEP plot is shown in Figure 2. The X-ray data allowed the relative configuration of brasilamide A to be determined as depicted in **1**. The presence of a relatively high percentage of oxygen in **1** should exhibit enough anomalous dispersion of Cu-K_α radiation to allow determination of its absolute configuration[20] with the Flack parameter value close to 0.0.[21] Therefore, the (1*S*,2*S*,5*R*,7*R*,9*S*) absolute configuration was proposed for **1** on the basis of the value of the Flack absolute structure parameter –0.02 (16).[21]

Brasilamide B (**2**) was assigned the elemental composition $C_{15}H_{23}NO_3$ (five degrees of unsaturation) by HRMS (ESI) analysis (*m*/*z* = 288.1559 [M + Na]$^+$, Δ = +1.1 mmu), 28 mass units less than **1**. The ^{1}H and ^{13}C NMR spectra of **2** displayed signals for structural features similar to those found in **1**, except that the α,β-unsaturated ketone (C-12; δ = 200.0 ppm) and the carboxy (C-3; δ = 172.6 ppm) carbon atoms in **1** are replaced by two methylenes (δ = 2.09/24.8 and 3.82, 3.86/69.4 ppm, respectively) in the spectra of **2**. These observations were supported by HMBC correlations from 11-H to the methylene carbon atoms C-3 and C-12. The relative and absolute configurations of **2** were deduced to be the same as those of **1** by comparison of their ^{1}H–^{1}H coupling constants and NOESY data. As confirmation, the absolute configuration of the C-5 tertiary alcohol in **2** was also assigned on the basis of circular dichroism of the in situ formed [$Rh_2(OCOCF_3)_4$] complex,[22] with the inherent contribution subtracted. Upon addition of [$Rh_2(OCOCF_3)_4$] to **2** in CH_2Cl_2 solution, a metal complex of the C-5 tertiary alcohol with [$Rh_2(OCOCF_3)_4$] was generated as an auxiliary chromophore. It was demonstrated that the sign of the E band (at ca. 350 nm) can be used to correlate the absolute configuration of a tertiary alcohol by applying the bulkiness rule.[22,23] In this case, the Rh complex of **2** displayed a positive E band (Figure 3), correlating to the (5*R*) absolute configuration, which is consistent with that assigned for **1**.

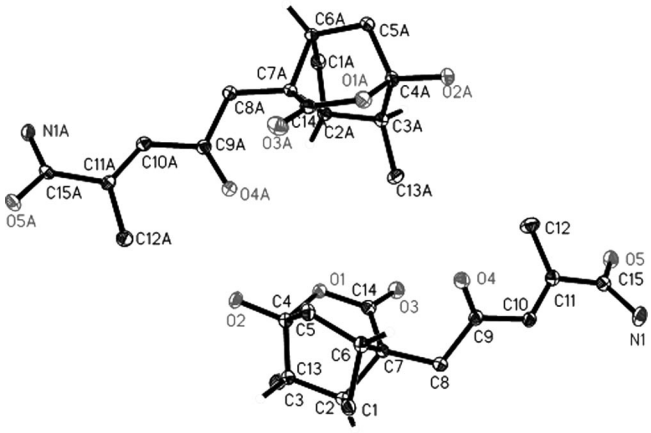


Figure 2. Thermal ellipsoid representation of **1**.

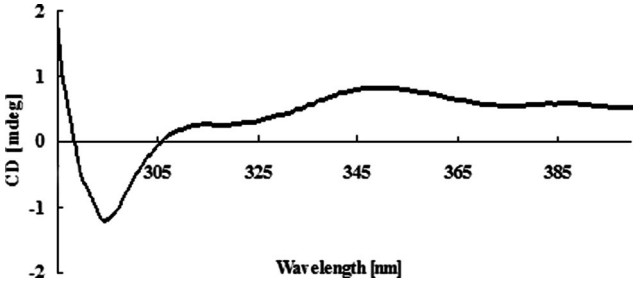


Figure 3. CD spectrum of the in situ formed Rh complex of **2** with the inherent CD spectrum subtracted.

Brasilamide C (**3**) gives a pseudomolecular ion [M + Na]$^+$ peak at *m*/*z* = 302.1359 (Δ = +0.4 mmu) in the HRMS (ESI) analysis, which corresponds to an elemental composition of $C_{15}H_{21}NO_4$ (six degrees of unsaturation). The ^{1}H and ^{13}C NMR spectroscopic data (Table 2) of **3** closely resemble those of known pinthunamide (**5**), except that the

Table 2. ^1H and ^{13}C NMR spectroscopic data of **2**–**4** in [D_6]acetone.

	2		**3**		**4**	
Pos.	δ_H [ppm] (mult., *J* [Hz])[a]	δ_C [ppm][b]	δ_H [ppm] (mult., *J* [Hz])[a]	δ_C [ppm][b]	δ_H [ppm] (mult., *J* [Hz])[a]	δ_C [ppm][b]
1	1.92 (t, 6.0)	44.8, CH		88.8, C_q		86.6, C_q
2		40.8, C_q	1.52 (m), 1.92 (m)	28.6, CH_2	1.60 (m), 1.90 (m)	28.6, CH_2
3	3.82 (d, 10), 3.86 (d, 10)	69.4, CH_2	1.75 (m), 1.97 (m)	23.3, CH_2	1.75 (m), 1.95 (m)	23.1, CH_2
4			2.18 (m)	41.1, CH	2.20 (m)	41.0, CH
5		97.1, C_q	1.46 (d, 10), 2.12 (ddd, 10, 6.0, 5.5)	23.3, CH_2	1.50 (d, 10), 2.15 (ddd, 10, 6.0, 5.5)	23.1, CH_2
6	1.81 (d, 12), 2.17 (dd, 12, 6.5)	42.8, CH_2	2.31 (dd, 5.5, 5.0)	48.3, CH	2.25 (dd, 5.5, 5.0)	49.1, CH
7	2.23 (m)	38.4, CH		53.6, C_q		53.5, C_q
8	1.14 (d, 10), 2.46 (ddd, 10, 7.0, 6.0)	37.0, CH_2	3.37 (d, 9.0), 4.08 (d, 9.0)	72.0, CH_2	3.39 (d, 9.0), 4.09 (d, 9.0)	72.1, CH_2
9	1.89 (q, 7.0)	45.1, CH				
10	1.02 (d, 7.0)	15.2, CH_3	3.42 (br. s)	67.3, CH_2	3.93 (d, 13), 4.02 (d, 13)	68.6, CH_2
11	1.64 (m)	32.8, CH_2	2.89 (d, 18), 3.29 (d, 18)	49.8, CH_2	2.92 (d, 18), 3.31 (d, 18)	49.7, CH_2
12	2.09 (m)	24.8, CH_2		201.4, C_q		201.2, C_q
13	6.38 (t, 6.5)	136.3, CH	6.86 (s)	128.8, CH	6.86 (d, 1.5)	128.8, CH
14		131.1, C_q		145.6, C_q		145.6, C_q
15		170.6, C_q		170.6, C_q		170.8, C_q
16	1.80 (s)	12.7, CH_3	2.16 (s)	14.8, CH_3	2.17 (d, 1.5)	14.8, CH_3
17						170.8, C_q
18					1.98 (s)	20.6, CH_3
OH-5	4.68 (s)					
OH-10			3.33 (br. s)			
NH_2-15	6.08 (br. s), 6.71 (br. s)		6.58 (br. s), 7.14 (br. s)		6.58 (br. s), 7.14 (br. s)	

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

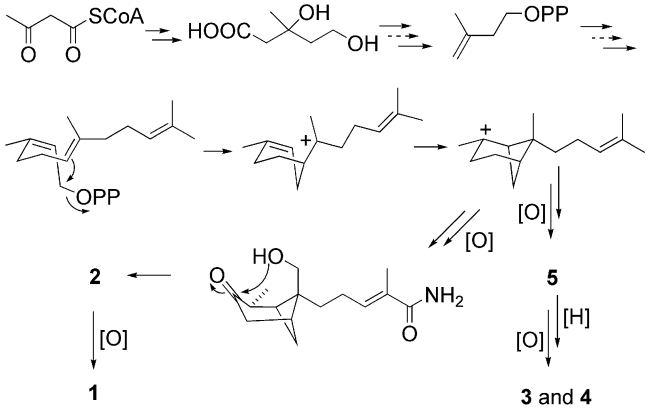
C-10 methyl group and the C-8 carboxy carbon atom are replaced by two oxygenated methylene units (δ = 3.42/67.3 and 3.37, 4.08/72.0 ppm, respectively) and one exchangeable proton (δ = 3.33 ppm) in the spectra of **3**. These observations were confirmed by HMBC correlations from 2-H and 6-H to C-10, 4-H and 11-H to C-8, 10-H to C-1 and C-6, and from 8-H to C-1, C-4, C-6, C-7, and C-11. The relative configuration of **3** was deduced to be the same as that of **5** by comparison of its ^1H–^1H coupling constants (Table 2) and NOESY data with those reported,[1] whereas the absolute configuration of **3** was presumed to be analogous to that of **5**, which was established by synthesis.[24]

The molecular formula of brasilamide D (**4**) was determined to be $C_{17}H_{23}NO_5$ (seven degrees of unsaturation) on the basis of its HRMS (ESI) spectrum (*m*/*z* = 344.1471 [M + Na]$^+$, Δ = –0.3 mmu), 42 mass units higher than that of **3**. Analysis of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **4** (Table 2) revealed nearly identical structural features to those found in **3**, except that the exchangeable proton (δ = 3.33 ppm) attached to C-10 is replaced by an acetyl group (δ = 1.98/20.6, 170.8 ppm) in the spectra of **4**. This observation was supported by the downfield shifts of 10-H (δ = 3.93, 4.02 ppm) and the HMBC correlation from 10-H to the carboxy carbon atom (δ = 170.8 ppm) of the acetyl group. Therefore, the structure of **4** was determined and its absolute configuration was deduced by analogy to **3**.

Brasilamides A–D (**1**–**4**) were tested for their in vitro activity against HIV-1, and compounds **2**–**4** displayed inhibitory effects on HIV-1 replication in C8166 cells, showing EC_{50} values of 108.8, 57.4, and 48.3 μM, respectively (all compounds showed CC_{50} values of greater than 200 μM; the positive control indinavir sulfate showed an EC_{50} value of 8.2 nM).

Conclusions

Brasilamides A–D (**1**–**4**) are new members of the bergamotane sesquiterpenoids. Compounds **1** and **2** are unique metabolites featuring a previously unknown 4-oxatricyclo[3.3.1.0^{2,7}]nonane skeleton, with a tetrahydro-2*H*-pyrone or a tetrahydro-2*H*-pyran moiety attached to the bicyclo[3.1.1]heptane ring at C-2 and C-5. Compounds **3** and **4** are new analogues of the known compound pinthunamide (**5**),[1] all possessing the unique 9-oxatricyclo[4.3.0.0^{4,7}]-nonane skeleton, but differ in having a tetrahydrofuran moiety attached to the bicycle[3.1.1]heptane unit rather than a γ-lactone ring and different substituents attached to C-10. Biogenetically, these metabolites could be derived through the mevalonate–*trans*-*cis*-farnesol–bisabolane–bergamotane pathway,[1] as proposed in Scheme 1. To the



Scheme 1. Proposed biogenesis for **1**–**4**.

best of our knowledge, compounds **1**–**5** are the first natural products to be reported from the plant endophytic fungus *P. brasiliense*.

Experimental Section

General: Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and UV data were recorded with a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded with a JASCO J-815 spectropolarimeter by using CH_2Cl_2 as the solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrometer. ^1H and ^{13}C NMR spectroscopic data were acquired with Varian Mercury-400 and –500 spectrometers using the solvent signals as references ([D$_6$]acetone: δ = 2.05/29.8, 206.1 ppm). HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. MS and HRMS (ESI) data were recorded with a Mariner ESI-TOF mass spectrometer.

Fungal Material: The culture of *Paraconiothyrium brasiliense* Verkley was isolated by one of the authors (L.G.) from branches of *Acer truncatum* Bunge on Dongling Mountain, Beijing, in March, 2005. The isolate was identified and assigned the accession number M3–3341 in the culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The isolate was subcultured on slants of potato dextrose agar (PDA) at 25 °C for 10 d. Agar plugs were used to inoculate in 250-mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 d. Fermentation was carried out in four 500-mL Fernbach flasks, each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 10^6 mL^{-1}. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 d.

Extraction and Isolation: The fermented material was extracted repeatedly with EtOAc (3 × 300 mL), and the organic solvent was evaporated to dryness under a vacuum to afford a crude extract (3.0 g) that was fractionated by silica gel vacuum column chromatography (CC) by using petroleum ether/EtOAc gradient elution. The fraction (99 mg) eluted with 100% EtOAc was subsequently separated by Sephadex LH-20 CC eluting with $CHCl_3$/MeOH (1:1). One subfraction (34 mg) was further purified by semi-preparative RPHPLC (Agilent Zorbax SB-C$_{18}$ column, 5 μM, 9.4 × 250 mm; 35% MeOH in H_2O for 2 min followed by 35–46% for 20 min; 2 mL min^{-1}) to afford brasilamide C (**3**; 6.0 mg, 0.2%, t_R = 16.50 min). The fractions eluted with 65 and 75% EtOAc were combined (202 mg) and fractionated again by Sephadex LH-20 CC using $CHCl_3$/MeOH (1:1) as eluent. Purification of the resulting subfractions by using different gradients afforded brasilamides A (**1**; 2.0 mg, 0.07%, t_R = 11.80 min; 30% MeOH in H_2O for 2 min followed by 30–60% for 20 min), B (**2**; 2.1 mg, 0.07%, t_R = 18.10 min; same gradient as in purification of **1**), and D (**4**; 4.0 mg, 0.13%, t_R = 24.20 min; 38% MeOH in H_2O for 2 min followed by 38–50% for 23 min).

Brasilamide A [(1′*S*,2′*S*,5′*R*,7′*R*,9′*S*,*E*)-5-(5′-Hydroxy-9′-methyl-3′-oxo-4′-oxatricyclo[3.3.1.0^{2,7}]non-2′-yl)-2-methyl-4-oxopent-2-enamide] (1): Colorless oil. $[\alpha]_D^{22}$ = –14 (*c* = 0.1, MeOH). UV (MeOH): λ (log ε, M^{-1} cm^{-1}) = 210 (4.11) nm. IR (neat): $\tilde{\nu}$ = 3350 (br.), 2967, 1709, 1669, 1601, 1321, 1218, 1110 cm^{-1}. For ^1H, ^{13}C NMR, and HMBC data, see Table 1. NOESY (400 MHz, [D$_6$]acetone, 25 °C): 1-H ↔ 10-H, 11-H; 7-H↔ 11-H; 8a-H ↔ 9-H; 8b-H ↔ 11-H; 9-H ↔ 8a-H; 10-H ↔ 1-H; 11-H ↔ 1-H, 7-H, 8b-H. HRMS (ESI): calcd. for $C_{15}H_{19}O_5Na$ [M + Na]$^+$ 316.1155; found 316.1147.

X-ray Crystallographic Analysis of 1: Upon crystallization from MeOH/H_2O (20:1) by using the vapor diffusion method, colorless crystals were obtained for **1** and a crystal (0.54 × 0.17 × 0.17 mm) was separated from the sample and mounted on a glass fiber, and data were collected by using a Rigaku R-AXIS RAPID IP diffractometer with graphite-monochromated Cu-K_a radiation (λ = 1.54186 Å) at 173(2) K. Crystal data: $C_{15}H_{19}NO_5$, *M* = 293.31, space group monoclinic, $P2_1$, unit cell dimensions: *a* = 10.0881(11) Å, *b* = 7.1849(8) Å, *c* = 19.8502(17) Å, *V* = 1397.3(2) Å^3, *Z* = 4, $D_{calcd.}$ = 1.394 mg m^{-3}, μ = 0.874 mm^{-1}, *F*(000) = 624. The structure was solved by direct methods by using SHELXL-97[25] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied using the Siemens Area Detector Absorption Program (SADABS).[26] The 16210 measurements yielded 4911 independent reflections after equivalent data had been averaged and Lorentz and polarization corrections applied. The final refinement gave R_1 = 0.0386 and wR_2 = 0.0882 [$I > 2\sigma(I)$].[27]

Brasilamide B (2): Colorless oil. $[\alpha]_D^{22}$ = –8.0 (*c* = 0.1, MeOH). UV (MeOH): λ (log ε, M^{-1} cm^{-1}) = 215 (4.14) nm. CD (*c* = 6.1 × 10^{-3} M, CH_2Cl_2): λ ($\Delta\varepsilon$) = 238 (–0.78), 289 (–0.92) nm. IR (neat): $\tilde{\nu}$ = 3350 (br.), 2931, 2927, 1707, 1670, 1594, 1452, 1377, 1056 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 2. HMBC (400 MHz, [D$_6$]acetone, 25 °C): 1-H → C-2, C-5, C-7, C-8, C-9, C-11; 3-H → C-1, C-2, C-5, C-7; 6-H → C-2, C-5, C-7, C-8, C-9; 7-H → C-1, C-2, C-5, C-8, C-11; 8a-H → C-1, C-2, C-3, C-6, C-7, C-9; 8b-H → C-1, C-6, C-7, C-9; 9-H → C-1, C-2, C-5, C-8; 10-H → C-1, C-5, C-9; 11-H → C-1, C-2, C-3, C-7, C-12, C-13; 12-H→ C-2, C-11, C-13, C-14; 13-H → C-11, C-12, C-15, C-16; 16-H → C-13, C-14, C-15; 5-OH → C-5, C-6, C-9. NOESY (500 MHz, [D$_6$]acetone, 25 °C): 1-H ↔ 10-H; 3-H ↔ 10-H, 11-H; 8a-H ↔ 9-H; 8b-H ↔ 11-H; 9-H ↔ 8a-H; 10-H ↔ 1-H, 3-H; 11-H ↔ 3-H, 8b-H. HRMS (ESI): calcd. for $C_{15}H_{23}NO_3Na$ [M + Na]$^+$ 288.1570; found 288.1559.

Absolute Conguration of the Tertiary Alcohol in 2:[22,23] According to the published procedure,[23] a sample of **2** (0.5 mg) was dissolved in a dry solution of the stock [$Rh_2(OCOCF_3)_4$] complex (0.8 mg) in CH_2Cl_2 (300 μL) and was subjected to CD measurements at a concentration of 1.7 mg mL^{-1}. The first CD spectrum was recorded immediately after mixing and its time evolution was monitored until stationary (ca. 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at around 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-5 tertiary alcohol moiety.

Brasilamide C (3): Colorless powder. $[\alpha]_D^{22}$ = +69 (*c* = 0.1, MeOH). UV (MeOH): λ (log ε, M^{-1} cm^{-1}) = 213 (4.17) nm. IR (neat): $\tilde{\nu}$ = 3350 (br.), 2934, 2869, 1751, 1669, 1603, 1380, 1194, 1083 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 2. HMBC data (400 MHz, [D$_6$]acetone, 25 °C): 2-H → C-1, C-4, C-6, C-10; 3-H → C-1, C-2, C-4, C-5, C-7; 4-H → C-2, C-3, C-5, C-6, C-7, C-8, C-11; 5a-H → C-1, C-4, C-6, C-7; 5b-H → C-1, C-3, C-4, C-6, C-7; 6-H → C-1, C-2, C-4, C-5, C-7, C-10, C-11; 8-H → C-1, C-4, C-6, C-7, C-11; 10-H → C-1, C-6; 11-H → C-4, C-6, C-7, C-8, C-12; 13-H → C-12, C-14, C-15, C-16; 16-H → C-13, C-14, C-15.

NOESY correlations (500 MHz, [D$_6$]acetone, 25 °C): 2b-H ↔ 5a-H; 3b-H ↔ 8b-H; 4-H ↔ 11b-H; 5a-H ↔ 2b-H; 5b-H ↔ 11b-H; 6-H ↔ 8a-H, 11b-H; 8a-H ↔ 6-H; 8b-H ↔ 3b-H; 11b-H ↔ 4-H, 5b-H, 6-H, 13-H; 13-H ↔ 11b-H. HRMS (ESI): calcd. for $C_{15}H_{21}NO_4Na$ $[M + Na]^+$ 302.1363; found 302.1359.

Brasilamide D (4): Colorless powder. $[\alpha]_D^{22} = +63$ ($c = 0.1$, MeOH). UV (MeOH): λ (logε, M^{-1}cm^{-1}) = 210 (4.15) nm. IR (neat): $\tilde{\nu}$ = 3347 (br.), 2929, 2879, 1736, 1669, 1604, 1369, 1243, 1038 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2. HMBC (400 MHz, [D$_6$]acetone, 25 °C): 2a-H → C-1, C-3, C-4, C-6; 2b-H → C-1, C-3, C-4, C-6, C-10; 3a-H → C-1, C-2, C-4, C-5; 3b-H → C-1, C-2, C-4, C-5, C-7; 4-H → C-2, C-3, C-5, C-6, C-7, C-11; 5a-H → C-1, C-3, C-4, C-6, C-7, C-8; 5b-H → C-1, C-3, C-4, C-6; 6-H → C-1, C-2, C-4, C-5, C-7, C-10, C-11; 8a-H → C-1, C-4, C-7, C-11; 8b-H → C-1, C-4, C-6, C-7; 10-H → C-1, C-2, C-6, C-17; 11a-H → C-4, C-6, C-7, C-8, C-12; 11b-H → C-4, C-6, C-7, C-8, C-12; 13-H → C-12, C-14, C-15, C-16; 16-H → C-13, C-14, C-15; 18-H → C-17. HRMS (ESI): calcd. for $C_{17}H_{23}NO_5Na$ $[M + Na]^+$ 344.1468; found 344.1471.

Anti-HIV Assays: Anti-HIV assays included cytotoxicity and HIV-1 replication inhibition evaluations.[28,29] Cells (3×10^4/well) were seeded into a 96-well microtiter plate in the absence or presence of various concentrations of test compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2. After incubation for 4 d, cell viability was measured by the MTT method. The concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined. In parallel with the MTT assay, a HIV-1 replication inhibition assay was determined by p24 antigen capture ELISA. C8166 cells were exposed to HIV-1 at 37 °C for 1.5 h, washed with PBS (phosphate-buffered saline) to remove free viruses and then seeded into a 96-well microtiter plate at 3×10^4 cells per well in the absence or presence of test compounds (indinavir sulfate was used as positive control). After 4 d, the supernatant was collected and inactivated by 0.5% Triton X-100. The supernatant was diluted three times, added to the plate coating with anti-p24 McAb (provided by Dr. Bin Yan, Wuhan Institute of Virology, Wuhan, People's Republic of China), and incubated at 37 °C for 1 h. After washing 5 times with PBST (phosphate-buffered saline with Tween-20), the HRP (horseradish peroxidase) labeled anti-p24 antibody (provided by Dr. Bin Yan) was added and incubated at 37 °C for 1 h. The plate was washed 5 times with PBST followed by adding OPD (*ortho*-phenylenediamine) reaction mixture. The assay plate was read at 490 nm by using a micro plate reader within 30 min. The EC_{50} values based on p24 antigen expression level were calculated.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of brasilamides A–D (**1**–**4**).

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[1] Y. Kimura, H. Nakajima, T. Hamasaki, F. Sugawara, L. Parkanyi, J. Clardy, *Tetrahedron Lett.* **1989**, *30*, 1267–1270.
[2] Y. Kimura, H. Nakajima, T. Hamasaki, T. Matsumoto, Y. Matsuda, A. Tsuneda, *Agric. Biol. Chem.* **1990**, *54*, 813–814.
[3] Y. Kimura, T. Matsumoto, H. Nakajima, T. Hamasaki, Y. Matsuda, *Biosci. Biotechnol. Biochem.* **1993**, *57*, 687–688.
[4] S. Nozoe, H. Kobayashi, N. Morisaki, *Tetrahedron Lett.* **1976**, *17*, 4625–4626.
[5] M. Massias, S. Rebuffat, L. Molho, A. Chiaroni, C. Riche, B. Bodo, *J. Am. Chem. Soc.* **1990**, *112*, 8112–8115.
[6] F. A. Macias, R. M. Varela, A. M. Simonet, H. G. Cutler, S. J. Cutler, R. A. Hill, *Tetrahedron Lett.* **2003**, *44*, 941–943.
[7] H. Oh, J. B. Gloer, C. A. Shearer, *J. Nat. Prod.* **1999**, *62*, 497–501.
[8] Y. Che, J. B. Gloer, B. Koster, D. Malloch, *J. Nat. Prod.* **2002**, *65*, 916–919.
[9] A. Chandra, L. N. Misra, R. S. Thakur, *Phytochemistry* **1987**, *26*, 3077–3078.
[10] N. B. Perry, E. J. Burgess, L. M. Foster, P. J. Gerard, *Tetrahedron Lett.* **2003**, *44*, 1651–1653.
[11] N. B. Perry, E. J. Burgess, L. M. Foster, P. J. Gerard, M. Toyota, Y. Asakawa, *J. Nat. Prod.* **2008**, *71*, 258–261.
[12] G. Strobel, B. Daisy, U. Castillo, J. Harper, *J. Nat. Prod.* **2004**, *67*, 257–268.
[13] G. Strobel, B. Daisy, *Microbiol. Mol. Bio. Rev.* **2003**, *67*, 491–502.
[14] R. Tan, W. Zou, *Nat. Prod. Rep.* **2001**, *18*, 448–459.
[15] G. Strobel, *Microbes Infect.* **2003**, *5*, 535–544.
[16] B. Schulz, C. Boyle, S. Draeger, A. K. Rommert, K. Krohn, *Mycol. Res.* **2002**, *106*, 996–1004.
[17] E. Li, R. Tian, S. Liu, X. Chen, L. Guo, Y. Che, *J. Nat. Prod.* **2008**, *71*, 664–668.
[18] G. Ding, L. Jiang, L. Guo, X. Chen, H. Zhang, Y. Che, *J. Nat. Prod.* **2008**, *71*, 1861–1865.
[19] L. Liu, S. Liu, X. Chen, L. Guo, Y. Che, *Bioorg. Med. Chem.* **2009**, *17*, 606–613.
[20] J. A. Beutler, K. L. McCall, K. Herbert, D. L. Herald, G. R. Pettit, T. Johnson, R. H. Shoemaker, M. R. Boyd, *J. Nat. Prod.* **2000**, *63*, 657–661.
[21] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.
[22] J. Frelek, W. J. Szczepek, *Tetrahedron: Asymmetry* **1999**, *10*, 1507–1520.
[23] M. Gerards, G. Snatzke, *Tetrahedron: Asymmetry* **1990**, *1*, 221–236.
[24] K. Mori, Y. Matsushima, *Synthesis* **1993**, *4*, 406–410.
[25] G. M. Sheldrick, *SHELXL-97*, *Program for X-ray Crystal Structure Solution and Refinement*, University of Göttingen, Göttingen, Germany, **1997**.
[26] G. M. Sheldrick, *SADABS*, *Program for Empirical Absorption Correction of Area Detector Data*, University of Göttingen, Göttingen, Germany, **1999**.
[27] CCDC-765974 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[28] L. Liu, R. Tian, S. Liu, X. Chen, L. Guo, Y. Che, *Bioorg. Med. Chem.* **2008**, *16*, 6021–6026.
[29] L. Liu, S. Liu, L. Jiang, X. Chen, L. Guo, Y. Che, *Org. Lett.* **2008**, *10*, 1397–1400.

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