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Heliconols A–C: Antimicrobial Hemiketals from the Freshwater Aquatic Fungus *Helicodendron giganteum*

Sanjay V. Mudur,[†] Dale C. Swenson,[†] James B. Gloer,*[,†] Jinx Campbell,[‡] and Carol A. Shearer[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

james-gloer@uiowa.edu

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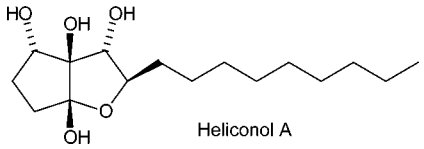
ABSTRACT

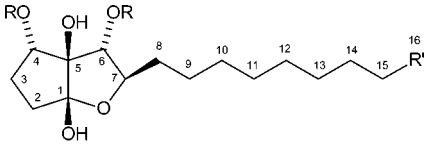


Heliconol A

Cultures of the freshwater aquatic fungus *Helicodendron giganteum* afforded three new compounds, heliconols A–C (1–3), that contain an unusual reduced furanocyclopentane unit. The structures of these metabolites were assigned by analysis of 1D and 2D NMR data. The absolute configuration of heliconol A (1) was assigned by single-crystal X-ray crystallographic analysis of its dibromobenzoate derivative. Heliconol A showed antifungal and antibacterial activities in disk diffusion assays.

In the course of our ongoing research on freshwater aquatic fungi,[1] we investigated an isolate (CS988-1B) of the freshwater aquatic fungus *Helicodendron giganteum* Glen-Bott (Helotiaceae) that was collected from a sample of submerged wood in Alaska. The genus *Helicodendron* is a member of the so-called helicosporous fungi[2]—an intriguing group of specialized aeroaquatic fungal genera that are widespread, but rarely studied. These fungi display a unique mode of adaptation in aquatic environments. They develop only mycelia underwater but sporulate when exposed above the surface, forming conidia that entrap air. The trapped air causes them to float, thereby aiding spore dispersal. To our knowledge, there have been no prior reports of chemistry from any *Helicodendron* species.

The isolate employed in this study was collected from a sample of submerged wood in Alaska using methods that have been described previously.[3] The ethyl acetate extract obtained from cultures of this isolate afforded three new polyketide-derived hemiketals (heliconols A−C (**1**−**3**)),[4] each of which contains an unusual reduced furanocyclopentane unit. Details of the isolation and structure elucidation of these compounds are reported here.



1 R = H; R' = CH_3
2 R = H; R' = CH_2OH
3 R = H; R' = COOH
4 R = *p*-C_6H_4BrCO; R' = CH_3

The molecular formula of heliconol A (**1**)[5] was determined to be $C_{16}H_{30}O_5$ on the basis of 1D NMR (Table 1) and HRESIMS data. Inspection of ^{13}C NMR and DEPT spectra of **1** revealed the presence of one methyl group, 10 aliphatic

[†] University of Iowa.
[‡] University of Illinois.

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Table 1. NMR Data for Heliconol A (1) Acquired in DMSO-d_6

position	δ_H (mult; J in Hz)[a]	δ_C[b,c]	HMBC (H#→C#)[a]
1		108.5	
2α	1.87 (ddd; 12, 6.7, 4.5)	35.1	1, 3, 4, 5
2β	1.59 (ddd; 12, 9.6, 6.8)		1, 3, 4, 5
3α	1.51 (m)	30.5	1, 2, 4
3β	1.80 (ddt; 12, 6.8, 4.5)		1, 2, 4, 5
4	4.05 (dt; 4.0, 6.8)	81.4	1, 2, 3, 5, 6
5		83.2	
6	3.73 (dd; 7.6, 6.8)	84.0	1, 4, 5, 7, 8
7	3.56 (dt; 4.4, 7.6)	80.1	1, 5, 6, 8, 9
8	1.51 (m), 1.38 (m)	34.1	6, 7, 9
9	1.38 (m), 1.25 (m)	25.3	
10−13[d]	1.25 (m)	29.1, 29.04	
		28.96, 28.7	
14	1.25 (m)	31.3	
15	1.25 (m)	22.1	
16	0.85 (t; 7.0)	14.0	14, 15
1-OH	5.84 (s)		1, 2, 5
4-OH	5.49 (d; 4.0)		3, 4, 5
5-OH	4.58 (s)		1, 4, 6[e]
6-OH	5.58 (d; 6.8)		6[e], 7

[a] 600 MHz. [b] 100 MHz. [c] Multiplicities were determined by a DEPT experiment and are consistent with the assignments. [d] These ^{13}C signals were not specifically assigned due to overlap of the corresponding proton resonances. [e] HMBC cross-peaks correlating to these carbons may also include correlation with the nearby signal for C-5.

methylenes, three sp^3 oxymethines, and two oxygenated quaternary sp^3 carbons, one of which is doubly oxygenated (δ 108.5). The molecular formula requires two unsaturations, but the absence of any sp^2 carbons indicated that **1** is bicyclic.

The ^1H NMR spectrum of heliconol A (**1**) contained a methyl triplet and a broad, intense signal centered at δ 1.25 suggestive of a methyl-terminated polymethylene chain. It also revealed signals for four exchangeable protons that must correspond to hydroxyl groups given the absence of nitrogen and carbonyl carbons. The multiplicities of these exchangeable proton signals (two doublets and two singlets) indicated that two of these hydroxy groups must be attached to methine carbons and the other two must be bonded to quaternary carbons. This further implied that the third oxymethine and the doubly oxygenated carbon (C-1) must be linked via the remaining oxygen atom to form a hemiketal moiety. ^1H NMR coupling data enabled identification of the two oxymethine carbons bearing the hydroxy groups (C-4 and C-6) and also led to recognition of a $CHCH_2CH_2$ spin system corresponding to the C2−C4 unit in **1**. The remainder of the structure of heliconol A (**1**) was ultimately assigned by analysis of HMBC and HMQC data.

HMBC correlations (Table 1) of the 1-OH to C-1 and C-5, of the 5-OH to C-1 and C-6, and of the 6-OH to C-7 established the presence of a tetrahydrofuran moiety. The fusion of a cyclopentane unit to the tetrahydrofuran ring was demonstrated by observation of cross-peaks from the 5-OH to C-4, from the 4-OH to C-3, C-4, and C-5, from H-4 to C-2 and C-3, and from the 1-OH to C-2. Since two of the 10 methylene groups were incorporated into the reduced furanocyclopentane ring system, it was clear that the remaining units must form a side chain containing the remaining eight methylene units and terminating with a methyl group. The only position available for connectivity of this side chain to the ring system was at oxymethine C-7, and this connection was confirmed by HMBC correlations of H-7 to C-8 and C-9 and of H-6 to C-8.

The relative configuration of heliconol A (**1**) was proposed by analysis of NOESY correlations (Figure 1). The assignment proved to be straightforward mainly due to relevant correlations exhibited by the hydroxyl protons. Strong correlations of both the 5-OH and the 1-OH signals with H-4 and H-6 placed all of these protons on the β-face of the ring system. Correlations of both the 6-OH and the 4-OH with H-7 indicated that these three protons are on the opposite (α) face of the ring system. Additionally, differential NMR assignments of the methylene protons at C-2 and C-3 were made possible by correlations of H-7 with H-2α and of the 4-OH with H-3α.

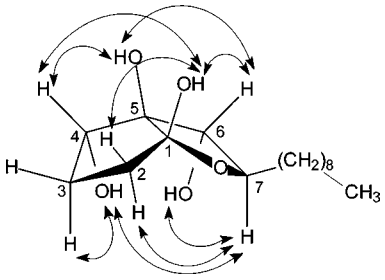

Figure 1. Key NOESY correlations observed for heliconol A (**1**).

(4) A subculture of this isolate is deposited at the Department of Plant Biology, University of Illinois Fungal Collection, with the accession number CS988-1B. Ascospores were subcultured onto 250 g of rice and incubated at 25 °C under 12 h light/12 h dark conditions. After 5 weeks, the fermentation mixture was broken up with a spatula and extracted with EtOAc (2 × 250 mL). The extracts were combined and filtered, and the resulting EtOAc-soluble filtrate was evaporated to obtain the crude extract (740 mg) that was partitioned between CH_3CN and hexanes. The CH_3CN-soluble portion (685 mg) was then fractionated through a column packed with silica gel and eluted with a CH_2Cl_2−MeOH step gradient (100%, 98%, 97%, 96%, 95%, 94%, 92%, 90%, 87%, 85%, and 80%) to afford 11 fractions. The ninth fraction (198 mg; eluted with 87% CH_2Cl_2) was further subjected to Sephadex LH-20 column chromatography, eluting successively with CH_2Cl_2−hexanes (4: 1, 90 mL), CH_2Cl_2−hexanes−acetone (4:1:1, 30 mL), and CH_2Cl_2−acetone (3:2, 30 mL; 2:3, 50 mL; 1:4, 100 mL) to afford heliconol A (**1**; 66 mg). Fraction 10 obtained from silica gel column chromatography (124 mg) was further fractionated through a Sephadex LH-20 column using varied proportions of CH_2Cl_2−acetone (9:1, 50 mL; 4:1, 70 mL; 1:1, 80 mL; 1:4, 50 mL; 100% acetone). A fraction that eluted with 1:1 CH_2Cl_2−acetone (98 mg) was again fractionated through Sephadex LH-20 using CH_2Cl_2−acetone (3:2, 70 mL; 1:1, 50 mL; 100% acetone) to afford heliconol B (**2**; 31 mg). Silica gel column fraction 11 (81 mg) was also subjected to Sephadex LH-20 column chromatography, eluting with CH_2Cl_2−acetone (4:1, 50 mL; 1:1, 80 mL; 1:4, 100 mL; 100% acetone). Impure heliconol C (**3**) eluted with 1:4 CH_2Cl_2−acetone as fractions 9 (10 mg) and 10 (19 mg). Further purification was carried out by subjecting the somewhat cleaner fraction 10 to Sephadex LH-20 column chromatography using CH_2Cl_2−acetone (1:1, 30 mL; 2:3, 30 mL; 1:4, 50 mL) to afford heliconol C (**3**; 3.3 mg).

(5) Heliconol A (**1**): colorless oil; $[\alpha]_D$ + 21 (*c* 1.6, acetone); ^1H NMR, ^{13}C NMR, and HMBC data, see Table 1; NOESY data (DMSO-d_6) 1-OH ↔ H-2α, 2β, 4, 6; 4-OH ↔ H-2α, 3α, 3β, 4, 6, 7; 5-OH ↔ 2β, 3β, 4, 6; 6-OH ↔ H-4, 6, 7; H-6 ↔ H-8a; H-7 ↔ H-2α; HRESIMS obsd *m/z* 325.1994 $(M + Na)^+$, calcd for $C_{16}H_{30}O_5Na$, 325.1991.

The molecular formula of heliconol B (**2**)[6] was assigned as $C_{16}H_{30}O_6$ on the basis of its ^{1}H and ^{13}C NMR and HRESIMS data. The ^{1}H NMR spectrum of **2** was nearly identical to that of heliconol A (**1**) except for three significant differences. The terminal methyl triplet observed for **1** was absent in the ^{1}H NMR spectrum of heliconol B (**2**). Instead, the ^{1}H NMR spectrum of **2** revealed two resonances not observed for **1** at δ 4.30 (1H, t, exchangeable) and δ 3.37 (2H, dt) that were coupled to each other ($^3J_{H-H} = 5.2$ Hz). These observations, together with retention of the carbon count and the inclusion of one more oxygen in **2** than in **1**, suggested replacement of the methyl group with a hydroxymethylene unit. This premise was supported by observation of an oxymethylene resonance at δ 60.7 in the ^{13}C NMR and DEPT spectra of **2**. Thus, the structure of heliconol B (**2**) was assigned as shown.

The molecular formula of heliconol C (**3**)[7] was assigned as $C_{16}H_{28}O_7$ (three degrees of unsaturation) by analysis of its NMR and HRESIMS data. Again, the NMR data were nearly identical to those of **1** and **2**. However, the ^{1}H NMR spectrum of **3** showed a two-proton triplet at δ 2.18 that was absent in the spectra for **1** and **2**. This observation, together with the molecular formula, the absence of methyl or hydroxymethylene signals, and observation of a new carbon resonance at δ 174.5 suggested the presence of a carboxylic acid group in place of the terminal methyl present in heliconol A (**1**). Thus, the structure of heliconol C (**3**) was assigned as shown.

In an effort to determine the absolute configuration of heliconol A (**1**), a dibromobenzoate derivative of **1** was prepared by treatment with *p*-dibromobenzoyl chloride.[8] Given the proximity of the resulting chromophores positioned in a 1,3-pseudovicinal syn orientation, it was proposed that some interaction of the chromophores might occur that could lead to assignment of the absolute stereochemistry by analysis of CD data. However, the CD spectrum of dibromobenzoate (**4**) contained only a monosignate curve and did not lend itself to application of the exciton chirality method. Fortunately, crystals of **4** were obtained from $CH_3CN-EtOH$ and the absolute configuration of **1** was assigned by single-crystal X-ray crystallographic analysis of **4** utilizing the anomalous dispersion effect induced by the bromine atoms. Thus, the X-ray structure derived from crystallographic analysis (Figure 2) not only confirmed the relative stereostructure assigned

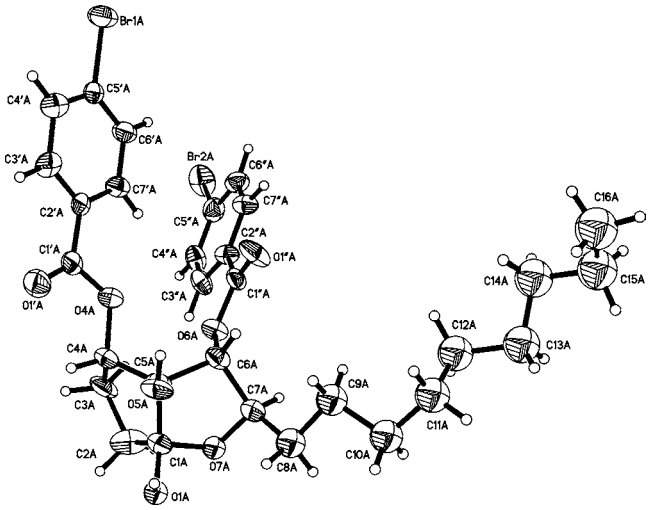

Figure 2. X-ray Model of 4,6-bis(4-bromobenzoyl)heliconol A (**4**).

by analysis of NOESY data, but also enabled assignment of the absolute configuration of heliconol A (**1**) as shown (1*R*,4*S*,5*S*,6*R*,7*R*).[9] Since the ring structures and the func-

(6) Heliconol B (**2**): colorless glass; $[\alpha]_D + 21$ (*c* 0.3, acetone); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 5.83 (s, 1-OH), 5.57 (d, $J = 6.6$ Hz, 6-OH), 5.49 (br d, $J = 3.6$ Hz, 4-OH), 4.58 (s, 5-OH), 4.30 (t, $J = 5.2$ Hz, 16-OH), 4.05 (br dt, $J = 3.6$, 6.8 Hz, H-4), 3.74 (dd, $J = 7.6$, 6.6 Hz, H-6), 3.56 (dt, $J = 4.4$, 7.6 Hz, H-7), 3.37 (dt, $J = 5.2$, 6.5 Hz, H_2-16), 1.87 (ddd, $J = 12$, 6.8, 4.5 Hz, H-2α), 1.80 (ddt, $J = 12$, 6.8, 4.5 Hz, H-3β), 1.59 (ddd, $J = 12$, 9.6, 6.8 Hz, H-2β), 1.51 (m, H-3α, H-8a), 1.39 (4H, m, H-8b, H-9a, H_2-15), 1.25 (11H, br s, H-9b, H_2-10, H_2-11, H_2-12, H_2-13, H_2-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 108.5 (C, C-1), 84.0 (CH, C-6), 83.2 (C, C-5), 81.4 (CH, C-4), 80.1 (CH, C-7), 60.7 (CH_2, C-16), 35.1 (CH_2, C-2), 34.1 (CH_2, C-8), 32.5 (CH_2, C-15), 30.5 (CH_2, C-3), 29.1, 29.04, 29.0, 28.9 (4CH_2, C-10, C-11, C-12, C-13), 25.5 (CH_2, C-14), 25.3 (CH_2, C-9); HRESIMS obsd *m*/*z* 341.1947 $(M + Na)^+$, calcd for $C_{16}H_{30}O_6Na$, 341.1940.

(7) Heliconol C (**3**): colorless glass; $[\alpha]_D +25$ (*c* 0.1, acetone); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 4.04 (t, $J = 6.8$ Hz, H-4), 3.74 (d, $J = 7.6$ Hz, H-6), 3.56 (dt, $J = 4.4$, 7.6 Hz, H-7), 2.18 (t, $J = 7.4$ Hz, H_2-15), 1.87 (ddd, $J = 12$, 6.7, 4.5 Hz, H-2α), 1.80 (ddt, $J = 12$, 6.8, 4.5 Hz, H-3β), 1.59 (ddd, $J = 12$, 9.6, 6.8 Hz, H-2β), 1.50 (4H, m, H-3α, H-8a, H_2-14), 1.38 (m, H-8b, H-9a), 1.25 (9H, m, H-9b, H_2-10, H_2-11, H_2-12, H_2-13); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.5 (C, C-16), 108.5 (C, C-1), 84.0 (CH, C-6), 83.2 (C, C-5), 81.4 (CH, C-4), 80.1 (CH, C-7), 35.1 (CH_2, C-2), 34.0 (CH_2, C-8), 33.7 (CH_2, C-15), 30.5 (CH_2, C-3), 29.1, 28.9, 28.7, 28.5 (4CH_2, C-10, C-11, C-12, C-13), 25.3 (CH_2, C-9), 24.5 (CH_2, C-14); HRESIMS obsd *m*/*z* 331.1769 $(M - H)^-$, calcd for $C_{16}H_{27}O_7$, 331.1757.

(8) A sample of **1** (5.6 mg, 0.018 mmol), *p*-bromobenzoyl chloride (9.1 mg; 0.042 mmol), and DMAP (8.9 mg; 0.073 mmol) were dissolved in anhydrous CH_2Cl_2 (4 mL), and the solution was stirred at room temperature under Ar. TLC analysis of the solution after 20 h indicated near completion of the reaction. The solution was combined with saturated aqueous $NaHCO_3$ solution (2 mL) and H_2O (1 mL), and the resulting mixture was extracted with CH_2Cl_2 (4 × 2 mL). The combined organic extract was dried under airflow, and the residue was subjected to reversed-phase HPLC with CH_3CN-H_2O gradient elution (75% CH_3CN over 20 min, 75−100% CH_3CN over 20 min, and 100% CH_3CN over 48 min) at 2.0 mL/min and UV detection at 215 nm to afford pure 4,6-bis(4-bromobenzoyl)heliconol A (**4**; 2.1 mg; $t_R = 57$ min) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (2H, d, $J = 8.7$ Hz), 7.53 (2H, d, $J = 8.7$ Hz), 7.46 (2H, d, $J = 8.7$ Hz), 7.42 (2H, d, $J = 8.7$ Hz), 5.60 (s, 1-OH), 5.24 (t, $J = 7.2$ Hz, H-4), 5.14 (d, $J = 5.0$ Hz, H-6), 4.18 (s, 5-OH), 4.13 (ddd, $J = 7.8$, 5.9, 5.0 Hz, H-7), 2.41 (1H, m), 2.13 (3H, m), 1.69 (2H, m), 1.25 (14H, m), 0.85 (t, $J = 6.9$ Hz, H_3-16); ESIMS obsd *m*/*z* 689 $(M + Na)^+$, 691 $[(M + 2) + Na]^+$.

(9) A colorless plate (0.17 × 0.13 × 0.025 mm) of 4,6-bis(4-bromobenzoyl)heliconol A (**4**) was taken from a sample that was obtained by crystallization from $CH_3CN-EtOH$. The crystal was mounted with grease on the tip of a glass capillary epoxied to a brass pin and placed on the diffractometer with the long crystal dimension (unit cell *b*-axis) approximately parallel to the diffractometer φ axis. Data for **4** were collected on a Nonius Kappa CCD diffractometer (Mo Kα radiation; graphite monochromator) at 190(2) K (cold N_2 gas stream) using standard CCD techniques yielding 15483 data. Lorentz and polarization corrections were applied. A correction for absorption using the multiscan technique was applied ($T_{max} = 0.9332$, $T_{min} = 0.6472$). Equivalent data were averaged yielding 5107 unique data [$R_{int} = 0.093$, 4129 $F > 4\sigma(F)$, Freidel pairs not averaged]. Based on a preliminary examination of the crystal, the space group *P*1 was assigned. The computer programs from the HKL package were used for data reduction. A preliminary model of the structure was obtained using XS, a direct methods program. Least-squares refining of the model vs the data was performed with XL computer program. Illustrations were made with the XP program and tables were made with the XCIF program. All are in the SHELXTL v6.1 package. Thermal ellipsoids shown in Figure 2 are at the 35% level. Most non-hydrogen atoms were refined with anisotropic thermal parameters. All H atoms were included with the riding model using the XL program default values. There are two

tionalities in **2** and **3** were identical to those in **1**, the absolute configurations of heliconols A and B (**2** and **3**) were presumed to be the same as that of heliconol A (**1**).

Heliconols A−C (**1**−**3**) were tested against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) in agar disk diffusion assays at 200 μg/disk.[10] Heliconol A (**1**) was found to inhibit the growth of *F. verticillioides*, affording a 15-mm diameter zone of partial clearing after 48 h, while compounds **2** and **3** showed no activity. Heliconols A−C (**1**−**3**) were also tested against *Candida albicans* (ATCC 14053), *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6051), and *Escherichia coli* (ATCC 25922) in standard disk assays.[11] Heliconol A (**1**) exhibited activity against *C. albicans*, *S. aureus*, and *B. subtilis* with clear inhibition zones measuring 18, 23, and 35 mm diameter, respectively, after 48 h at 100 μg/disk. A 10-mm zone of inhibition was observed for **1** after 48 h when tested against *C. albicans* at 25 μg/disk. Compounds **2** and **3** were inactive in these assays at the 100 μg/mL level.

Heliconols A−C (**1**−**3**) are clearly assembled from acetate units and can be considered as polyketides, but it is also possible that they could arise from a modified fatty acid precursor. The reduced furanocyclopentane ring system found in **1**−**3** is rare among natural products, and, to our knowledge, has only two recent precedents among fungal metabolites (communiols E and F),[12] although it does appear as a subunit of a few, more complex ring system structures.[13]

Acknowledgment. We thank the staff of the University of Iowa MS and NMR facilities for their assistance. Financial support for this project from the National Institutes of Health (GM 60600) is greatly appreciated.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of heliconols A−C (**1**−**3**), ^{1}H NMR spectrum of 4,6-bis(4-bromobenzoyl)heliconol A (**4**), and additional X-ray crystallographic data for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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independent molecules (one with atom labels assigned using an "A" suffix and the other using a "B" suffix) in the asymmetric unit that forms a H-bonded dimer. The "A" unit is arbitrarily shown in Figure 2. The final refinement gave $R_1 = 0.0624$ and w$R_2 = 0.1272$. The somewhat high R values are due to weak high-angle scattering by the thin crystal which is compounded by significant disorder that was observed in the crystal. Details of the observed disorder and the treatment thereof in data handling are provided in the Supporting Information. Data with $2\theta > 40°$ were excluded from the final cycles of refinement for this reason [<30% had $I > 3\sigma(I)$]. Crystallographic data for 4,6-bis(4-bromobenzoyl)heliconol A (**4**) have been deposited with the Cambridge Crystallographic Data Centre (deposition no. 604083). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or email: deposit@ccdc.cam.ac.uk).

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