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Short Note

Fosbergenone: 3-[2-(1,2,5,5-Tetramethyl-7-oxo-1,2,3,4,4*a*,5,6,7-octahydronaphthalen-1-yl)ethyl]-2,5-dihydrofuran-2-one

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Abstract: A new *ent*-halimane diterpenoid, fosbergenone (**1**) was isolated from the foliar bud exudate of *Gardenia fosbergii*. The structure of **1** was elucidated based on spectroscopic data including IR, 1D and 2D NMR, as well as high-resolution mass spectrometry.

Keywords: diterpenoid; fosbergenone; *Gardenia fosbergii*; 2D NMR; high-resolution mass spectrometry

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1. Introduction

The genus *Gardenia* comprises around 250 species of flowering plants belonging to the family Rubiaceae. Many of them are cultivated worldwide for their large, fragrant flowers [1]. *Gardenia fosbergii* is one of the two endemic *Gardenia* species found in Sri Lanka. It is a shrub distributed throughout the eastern sector of the island and found especially on coastal sandy scrubland and occasionally on rocky outcrops [2,3]. The yellow resinous exudate produced by the apical foliar buds of this species is harvested and used in traditional medicine in Sri Lanka as an antiseptic in wound treatment and as an incense to sanitize the air from pathogenic microbes and deter household insect pests. The resin is commercially available in Ayurvedic medicinal shops throughout Sri Lanka. A phytochemical investigation of the species' resinous exudate was conducted by a team of Sri Lankan chemists in the early 1980 s which led to the discovery of many poly-methoxylated flavonoids [4]. In this paper, we report the isolation and structural elucidation of a new *ent*-halimane diterpenoid, fosbergenone (**1**) (Figure 1) from the resinous bud exudate of *Gardenia fosbergii*.

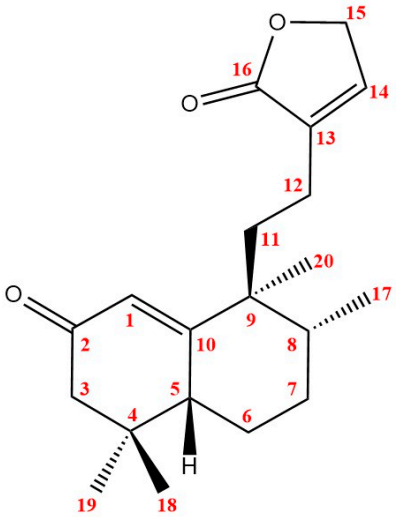

Figure 1. Structure of compound **1**.

2. Results

Extraction and Isolation

Foliar bud exudate (300 g) of *Gardenia fosbergii* was extracted three times with dichloromethane and methanol, for 24 h, at room temperature. The solvents were removed under reduced pressure to yield crude extracts of dichloromethane (50 g) and methanol (12.5 g), respectively. A portion (5.6 g) of the dichloromethane crude was fractionated by Medium Pressure Liquid Chromatography (MPLC) using a reversed-phase FlashPure C18 cartridge and eluted with MeOH/H_2O mobile phase, which afforded 8 subfractions (A–H). Subfraction C was subjected to High-Performance Liquid Chromatography (HPLC) separation, employing a reversed-phase C18 column and gradient MeOH/H_2O mobile phase which led to the isolation of compound **1** (28.6 mg).

Fosbergenone (**1**) yellow oil; QTOFMS *m*/*z* 317.2121 $[M + H]^+$ (calculated exact mass and error for $C_{20}H_{29}O_3$: *m*/*z* 317.2111 and −1.1 ppm); UV (MeOH) λ_{max}: 352, 246 and 210 nm; IR ν_{max}: 2960, 1750, 1665, 1455, 1370, and 1171 cm^{-1}; ^{1}H-NMR (CD_3OD-d_4, 400 MHz) and ^{13}C-NMR (CD_3OD-d_4 100 MHz) data of compound **1** are given in Table 1.

Table 1. ^{13}C and ^{1}H-NMR spectroscopic data of compound **1** in CD_3OD-d_4 at 100/400 MHz.

Position	δ_C/ppm, Type	δ_H/ppm (Integral, Multiplicity, *J*/Hz)
1	125.1, CH	5.8 (1H, *s*)
2	202.7, C	
3	49.6, CH_2	2.35 (1H, *d*, 15.5); 2.09 (1H, *d*, 15.5)
4	35.5, C	
5	46.4, CH	2.45 (1H, *dd*, 12.8, 4.7)
6	24.8, CH_2	1.86 (1H, *m*); 1.59 (1H, *m*)
7	29.2, CH_2	2.16 (1H, *m*); 1.47 (1H, *m*)
8	41.7, CH	1.84 (1H, *m*)
9	46.3, C	
10	173.1, C	
11	38.2, CH_2	2.30 (1H, *m*); 1.50 (1H, *m*)
12	21.4, CH_2	2.27 (1H, *m*); 1.92 (1H, *m*)
13	134.1, C	
14	148.0, CH	7.37 (1H, *s*)
15	72.2, CH_2	4.81 (2H, *s*)
16	176.9, C	
17	15.9, CH_3	0.87 (3H, *d*, 7.0)
18	28.6, CH_3	1.03 (3H, *s*)
19	25.9, CH_3	0.99 (3H, *s*)
20	21.8, CH_3	1.11 (3H, *s*)

3. Discussion

Compound **1** was obtained as a yellow oil from the HPLC subfraction with a reasonable yield. The molecular formula of **1** was established as $C_{20}H_{28}O_3$ with 7 degrees of unsaturation based on the LC-HRESI mass spectrum which displayed a base peak at *m*/*z* 317.2121 corresponding to the protonated molecular ion, $[M + H]^+$. The intense absorption bands observed at 210 and 246 nm in the UV spectrum of **1** are attributable to the permitted $\pi \rightarrow \pi^*$ transitions of conjugated carbonyl groups [5], while the weak band at 352 nm is characteristic of the forbidden $n \rightarrow \pi^*$ transition of a conjugated carbonyl functionality [5]. The bands exhibited by the IR spectrum could be assigned to the following bond vibrations: 2960 cm^{-1}: aliphatic sp^3 hybridized C-H stretching, 1750 cm^{-1}: carbonyl C=O stretching, 1665 cm^{-1}: alkene C=C stretching, 1455 and 1370 cm^{-1}: methyl group C–H bending and 1171 cm^{-1}: C–O stretching.

The ^{13}C-NMR spectrum of **1** revealed 20 carbon resonance signals which were classified as four methyls, six methylenes, four methines (2 olefinic and 2 sp^3) and six quaternary carbons (2 carbonyls, 2 olefinic and 2 sp^3), respectively, with the help of phase-sensitive HSQC 2D NMR data. The quaternary carbon resonance at δ_C 202.7 was assigned to a

keto-group due to its highly de-shielded position in the ^{13}C spectrum. Since compound **1** lacked any labile hydroxyl protons as evidenced in the HSQC spectrum (which indicated 28 protons), the next carbon resonance at δ_C 176.9 was assigned to an ester carbonyl and not a carboxylic acid. The 1D ^{1}H spectrum revealed a methine (H-14) singlet at δ_H 7.37 which was found to correlate with the de-shielded methylene (H-15) at δ_H 4.81 in the COSY data. This observation suggests that both H-14 and H-15 were part of a lactone bearing heterocycle. Strong correlations in the up-field region of the COSY revealed the presence of two spin systems in compound **1**. The first spin system was made-up by the couplings of H-5 (δ_H 2.45)/H-6 (δ_H 1.86, 1.59), H-6 (δ_H 1.86, 1.59)/H-7 (δ_H 2.16, 1.47), H-7 (δ_H 2.16, 1.47)/H-8 (δ_H 1.84) and H-8 (δ_H 1.84)/H-17 (δ_H 0.87) while the next comprised coupling between H-11 (δ_H 2.3, 1.5) and H-12 (δ_H 2.27, 1.92). The methine (H-1) singlet at δ_H 5.80 ppm was assigned to a trisubstituted alkene proton which was found to be weakly correlated to H-5 (δ_H 2.45) in the COSY, indicating an allylic coupling between the pair.

The strong HMBC correlations (Figure 2) observed between H-14 (δ_H 7.37) and C-16 (δ_C 176.9) and H-15 (δ_H 4.81) and C-16 (δ_C 176.9) revealed the presence of a (5H)-furan-2-one moiety in compound **1**. HMBC correlations between H-14 and C-12 and vice versa indicated the presence of a methylene side chain at C-13 position of the (5H)-furan-2-one moiety. The two methyl singlets (H-18, δ_H 1.03 and H-19, δ_H 0.99) found correlating identically with their surrounding carbons (C-3, C-4, C-5 and C-6) in the HMBC were identified as *gem*-dimethyls attached to the quaternary carbon C-4. Strong correlations in the HMBC (Figure 2) from H-3 to C-1 and C-2 confirmed the presence of an α,β-unsaturated ketone moiety in compound **1**. HMBC correlations from H-11 to C-9 and C-10 were used to confirm the linkage of the side chain (bearing (5H)-furan-2-one moiety) at carbon C-9 of the bicyclic ring system. COSY and HMBC correlations (Table S1, supplementary materials) were used to piece together and confirm an *ent*-halimane class diterpenoid scaffold for compound **1**. The experimental ^{13}C-NMR data of **1** were also compared with the reference data (Table 2) of two known halimane diterpenoids (**2** and **3**) to further validate the chemical shifts of **1**. Compound **2** shared a common bicyclic enone motif with **1** while **3** displayed a common diethylene side chain bearing (5H)-furan-2-one moiety with **1** (Figure 3). Comparison with the reference ^{13}C shifts revealed that **1** generally showed a similar trend in chemical shift differences (~1–2 ppm) with the corresponding carbons of the reference compounds. The reason for the slight variation in chemical shifts observed in **1** might be due to the different deuterated solvent (CD_3OD-d_4) in which it was dissolved.

Since the carbon chemical shifts of **1** were very similar to that of **2**, a NOESY experiment was performed on **1** to ascertain whether it shared the same relative stereochemistry as **2**. Strong NOE correlations between H-5 to H-11 and H-8 to H-11, H-12 confirmed that protons H-5, H-8 and the side chain bearing the conjugated (5H)-furan-2-one moiety were present on the same face of the bicyclic ring system. The larger coupling constant (12.8 Hz) between protons H-5 and either H-6 or H-6′, confirmed the axial position of H-5 on the ring and a strong NOE correlation from H-5 to H-18 verified that methyl group C-18 was in closer proximity (same face of the ring) to H-5 than its geminal partner C-19 which displayed no NOE correlation with H-5. The absence of a through-space correlation from H-5 to H-8 suggested that proton H-8 occupied an equatorial orientation on the ring. Furthermore, the axial orientation of the (5H)-furan-2-one-bearing side chain on the ring was confirmed by an NOE correlation between H-18 and H-12 and 3D molecular simulations which indicated that the axial conformer had shorter distances between its hydrogens (H-5 to H-11, H-8 to H-11 and H-18 to H-12) through space than its equatorial counterpart. (Figure 4). This evidence confirmed that compound **1** was an *ent*-halim-1(10)-ene class halimanolide diterpenoid [8]. Though more than 40 *ent*-halim-1(10)-enes have been discovered to date, only one halimanolide of this class has been reported so far [8] which makes this discovery significant.

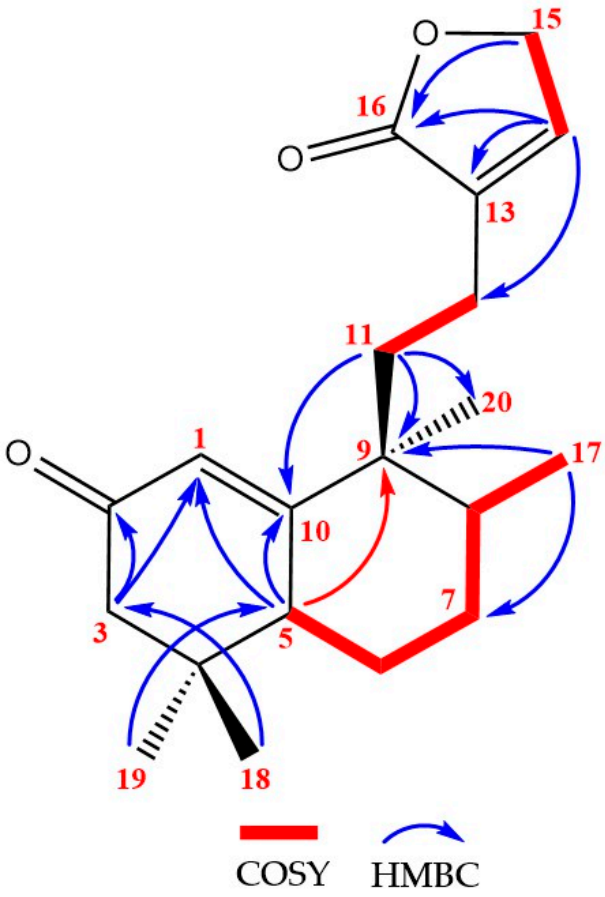

Figure 2. Key COSY and HMBC correlations of compound **1**.

Table 2. Reference ^{13}C-NMR chemical shifts of two known halimane diterpenoids with part of the scaffold of **1**.

Position	^{13}C Shifts of 1 in CD_3OD-d_4, Type	Reference ^{13}C Shifts of 2 in $CDCl_3$-d_1 [6], Type	Reference ^{13}C Shifts of 3 in $CDCl_3$-d_1 [7], Type
1	125.1, CH	122.8, CH *	18.3, CH_2
2	202.7, C	199.9, C *	23.7, CH_2
3	49.6, CH_2	48.5, CH_2 *	76.0, CH
4	35.5, C	34.5, C *	37.9, C
5	46.4, CH	45.3, CH *	86.4, C
6	24.8, CH_2	23.8, CH_2 *	29.7, CH_2
7	29.2, CH_2	34.0, CH_2 *	28.9, CH_2
8	41.7, CH	40.3, CH *	33.4, CH
9	46.3, C	44.9, C *	55.3, C
10	173.1, C	169.9, C *	46.3, CH
11	38.2, CH_2	37.8, CH_2 *	24.2, CH_2 *
12	21.4, CH_2	28.3, CH_2 *	19.5, CH_2 *
13	134.1, C	139.8, C	133.5, C *
14	148.0, CH	124.4, CH	144.1, CH *
15	72.2, CH_2	59.3, CH_2	70.4, CH_2 *
16	176.9, C	15.6, CH_3	174.0, C *
17	15.9, CH_3	21.5, CH_3 *	16.0, CH_3
18	28.6, CH_3	25.9, CH_3 *	23.9, CH_3
19	25.9, CH_3	28.5, CH_3 *	20.1, CH_3
20	21.8, CH_3	17.8, CH_3 *	177.5, C
Ac			170.6, C 21.1, CH_3

* Carbon chemical shifts belonging to part of the scaffold of **1.**

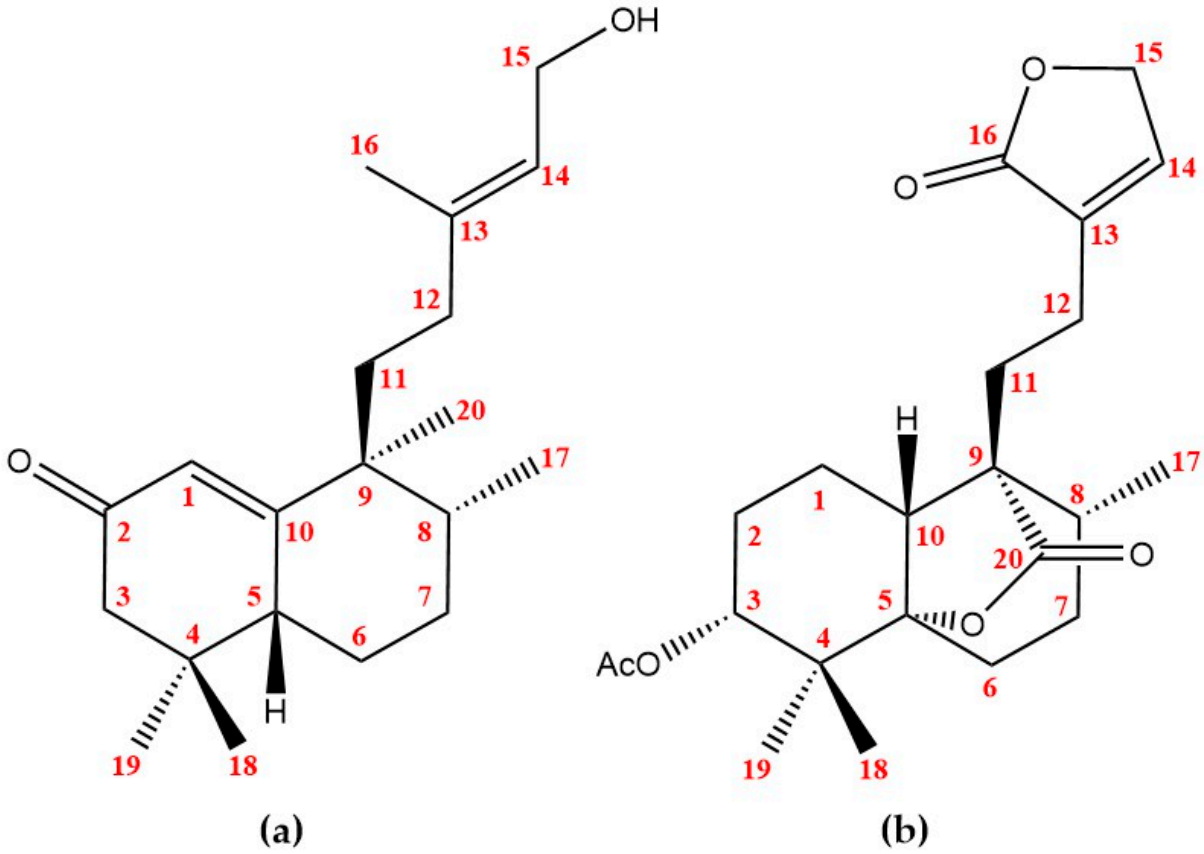

Figure 3. Structures of reference halimane diterpenoids displaying red coloured moieties common to **1**. (**a**) 2-oxo-friedolabd-1(10),13*E*-dien-15-ol; (**b**) acetic acid (1*S*,3*R*,6*R*,7*R*,8*R*)-2,2,8-trimethyl-12-oxo-7-[2-(2-oxo-2,5-dihydro-furan-3-yl)-ethyl]-11-oxa-tricyclo [5.3.2.01,6]dodec-3-yl ester.

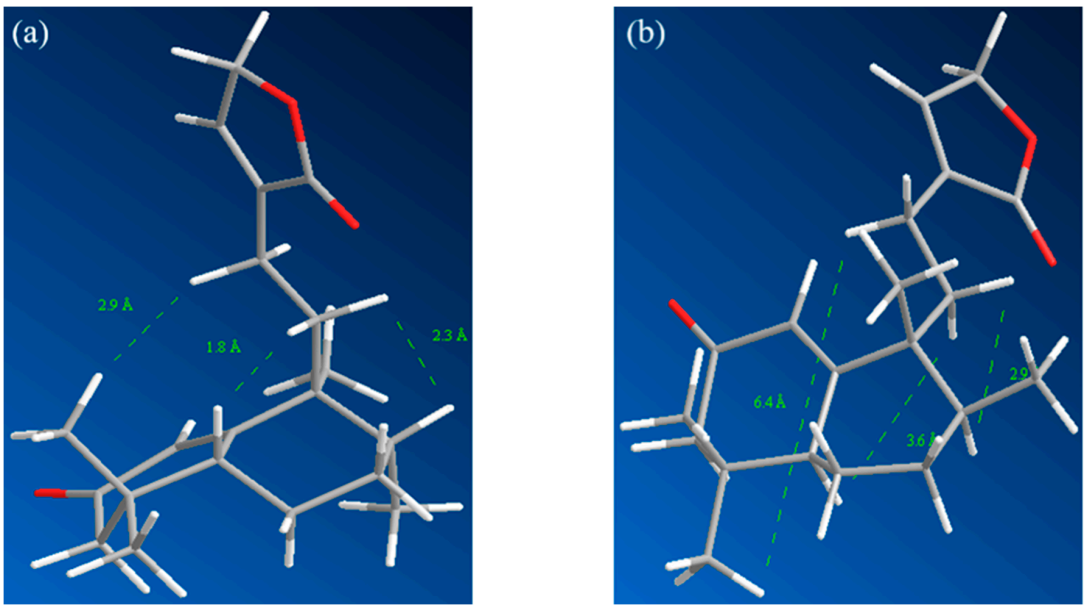

Figure 4. Three dimensional representation of compound **1**. (**a**) Axial orientation of the (5H)-furan-2-one bearing side chain on the ring; (**b**) Equatorial orientation of the (5H)-furan-2-one bearing side chain on the ring.

4. Materials and Methods

4.1. General Experimental Procedures

All organic solvents involved in the extraction and chromatography steps were purchased from Fisher Scientific Ltd. (Loughborough, UK) and used without further purification. Chromatographic fractionation was carried out on a Reveleris preparative flash system equipped with a reversed-phase EcoFlex C18 cartridge (80 g, 50 μm) and a mobile phase starting with 85% MeOH/15% H_2O for 5 min followed a by a linear gradient elution scheme to 100% MeOH/0% H_2O for 55 min which continued for a further 10 min at a flow rate of 10 mL/min. HPLC isolation was performed on an Agilent HPLC system (1200 series) equipped with a binary pump, diode array detector (G1315B), Waters SunFire C18 reversed-phase column (10 μ, 10 × 250 mm) and eluted with 80% MeOH/20% H_2O followed by a linear gradient elution scheme to 100% MeOH/0% H_2O for 33 min at a flow rate of 2 mL/min. Mass spectra were measured in the positive ion mode electrospray ionization using an MS system (Bruker MAXIS II equipped with a Quadrupole-Time-of-Flight mass analyser) coupled to an HPLC (Agilent 1290 Infinity equipped with a diode array detector) equipped with a Phenomenex analytical C18 column (2.5 μm, 100 Å, 4.6 × 150 mm), and eluted with a starting mobile phase of 5% ACN/95% H_2O (containing 0.1% formic acid) followed by a gradient of up to 100% ACN/0% H_2O (containing 0.1% formic acid) for 15 min, at a flow rate of 1 mL/min. The UV–visible spectrum was obtained from the Agilent HPLC system (1200 series) equipped with a diode array detector (G1315B). The IR spectrum was recorded using a Perkin Elmer Spectrum Two FT-IR spectrometer equipped with an ATR diamond cell. NMR spectra were recorded on a Bruker AVANCE III spectrometer in CD_3OD-d_4 (Thermo Fisher Scientific, Geel, Belgium) at 400 MHz for ^1H experiments and 100 MHz for ^{13}C experiments. All chemical shifts were reported in the standard δ notation of parts per million using the residual non-deuterated solvent peak (3.31 and 49.1 ppm for CD_3OD-d_4) as the reference. Then, 3-D structural simulations were performed with Chem3D 15.1 software.

4.2. Plant Material

Freshly collected bud exudate of *Gardenia fosbergii* was specially ordered and purchased from an Ayurvedic medicinal shop in Batticaloa Sri Lanka in October 2021. A material transfer agreement was signed between Rasiah Rajakulendran and the forest department of Sri Lanka to export 300 g of *Gardenia fosbergii* resin to the University of Aberdeen, UK, in October 2021. An export permit (Ref No. FD/EX/50/Nor/2021/10/75) and phytosanitary certificate (Ref No. 0369528) was obtained from the Department of Forest Conservation, Sri Lanka, and Department of Agriculture, Sri Lanka, respectively, for the shipment of the resin.

5. Conclusions

A new *ent*-halimane diterpenoid, fosbergenone (**1**) was isolated from the foliar bud exudate of *Gardenia fosbergii*. To the best of our knowledge, this is the first time a halimane diterpenoid has ever been reported from the genus *Gardenia* and plant family Rubiaceae.

Supplementary Materials: The following supporting information can be downloaded online. Figure S1: Annotated mass spectrum of compound **1**; Figure S2: Annotated UV spectrum of compound **1**; Figure S3: IR spectrum of compound **1**; Figure S4: Annotated ^1H-NMR spectrum of compound **1** in CD_3OD-d_4 at 400 MHz; Figure S5: Annotated ^{13}C-NMR spectrum of compound **1** in CD_3OD-d_4 at 100 MHz; Figure S6: HSQC spectrum of compound **1** in CD_3OD-d_4; Figure S7: COSY spectrum of compound **1** in CD_3OD-d_4; Figure S8: HMBC spectrum of compound **1** in CD_3OD-d_4: Figure S9: 2D NOESY spectrum of compound **1** in CD_3OD-d_4. Table S1. COSY and HMBC correlations of compound **1**.

Author Contributions: Conceptualization, J.E.R.; methodology, J.E.R.; software, J.E.R. and M.J.; investigation, data curation, J.E.R.; writing—original draft preparation, J.E.R.; writing—review and editing, J.E.R., E.T.O., M.J. and R.E.; supervision, M.J. and R.E.; funding acquisition, J.E.R. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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