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Antibacterial Acylphloroglucinols from *Hypericum olympicum*

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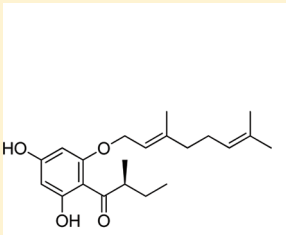
Supporting Information

ABSTRACT: New antibacterial acylphloroglucinols (**1**−**5**) were isolated and characterized from the aerial parts of the plant *Hypericum olympicum* L. cf. *uniflorum*. The structures of these compounds were confirmed by extensive 1D- and 2D-NMR experiments to be 4,6-dihydroxy-2-*O*-(3″,7″-dimethyl-2″,6″-octadienyl)-1-(2′-methylbutanoyl)benzene (**1**), 4,6-dihydroxy-2-*O*-(7″-hydroxy-3″,7″-dimethyl-2″,5″-octadienyl)-1-(2′-methylbutanoyl)benzene (**2**), 4,6-dihydroxy-2-*O*-(6″-hydroxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene (**3**), 4,6-dihydroxy-2-*O*-(6″-hydroperoxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene (**4**), and 4,6-dihydroxy-2-*O*-(6″,7″-epoxy-3″,7″-dimethyloct-2″-enyl)-1-(2′-methylbutanoyl)benzene (**5**). These new natural products have been given the trivial names olympicins A−E (**1**−**5**). All compounds were evaluated against a panel of methicillin-resistant *Staph. aureus* and multidrug-resistant strains of *Staph. aureus*. Compound **1** exhibited minimum inhibitory concentrations (MICs) of 0.5−1 mg/L against the tested *Staph. aureus* strains. Compounds **2** to **5** were also shown to be active, with MICs ranging from 64 to 128 mg/L. Compound **1** was synthesized using a simple four-step method that can be readily utilized to give a number of structural analogues of **1**.

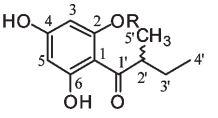


Olympicin A (1)

The widespread interest in the use of *Hypericum perforatum* (St. John's Wort) in mild to moderate depression has attracted much attention in investigating the bioactive metabolites from other species in the *Hypericum* genus. The acylphloroglucinol hyperforin is the best characterized acylphloroglucinol from this genus in terms of its bioactivity and has been shown to be the major antibacterial constituent in *H. perforatum*[1] and active against *Staph. aureus* and MRSA strains at concentrations as low as 0.1 mg/L.[2] Other acylphloroglucinol derivatives with antibacterial activities have been isolated and characterized from this genus.[3,4] Notable among these acylphloroglucinols are the antibacterial drummondins from *Hypericum drummondii*, which are filicinic acid derivatives displaying MIC values as low as 0.39 mg/L.[5] This study is part of ongoing research in our group to identify antibacterial metabolites from various *Hypericum* species.

These levels of activity, coupled with interesting acylphloroglucinol chemistry, which is often complex, prompted us to screen a range of *Hypericum* species collected from the UK National Collection at the Royal Botanic Gardens Kew at Wakehurst Place.[8] This led to the isolation and evaluation of the antibacterial properties of a series of new acylphloroglucinol natural products (**1**−**5**) from *Hypericum olympicum*. This species is a small herb with typical yellow *Hypericum* flowers.

The simplicity of compound **1**, coupled with its level of activity against a range of methicillin-resistant *Staph. aureus* (MRSA) and multidrug-resistant (MDR) strains of *Staph. aureus*, shows the value of this class as antibacterial drug leads, which could be further developed into antibiotics to treat resistant strains.



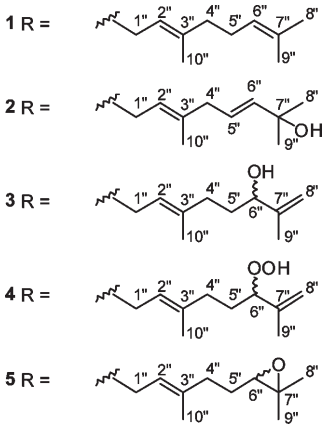


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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data and $^{1}H-^{13}C$ Long-Range Correlations for 1−3 Recorded in $CDCl_3$

	1				2				3			
position	^{1}H (J, Hz)	^{13}C	^{2}J	^{3}J	^{1}H (J, Hz)	^{13}C	^{2}J	^{3}J	^{1}H (J, Hz)	^{13}C	^{2}J	^{3}J
1		105.0				105.9				105.9		
2		162.6				162.4				162.4		
3	5.92 d (2.5)	91.5	C-4	C-1, C-5	5.90 d (2)	91.7	C-4	C-1, C-5	5.92 d (2)	91.6		C-1
4		161.9				162.1				161.9		
5	5.98 d (2.5)	96.5	C-6	C-1, C-3	5.99 d (2)	96.6	C-6	C-1, C-3	5.98 d (2)	96.6		C-3
6		167.5				167.5				167.6		
1′		210.4				210.3				210.3		
2′	3.66 m	46.1			3.63 m	46.1			3.64 m	46.1		
3′	1.37 m, 1.80 m	26.8			1.37 m, 1.80 m	26.9			1.37 m, 1.80 m	26.8		
4′	0.89 t (7.5)	11.8	C-3′	C-2′	0.88 t (7.5)	11.9	C-3′	C-2′	0.88 t (7.5)	11.9	C-3′	C-2′
5′	1.12 d (6.5)	16.6	C-2″	C-1′, C-3′	1.12 d (7)	16.7	C-2′	C-1′, C-3′	1.12 d (6.5)	16.7	C-2′	C-1′, C-3′
1″	4.57 d (6.5)	65.7	C-2″	C-3, C-3″	4.58 d (6.5)	65.6	C-2″	C-3″	4.57 d (6.5)	65.6		C-3″
2″	5.51 m	118.2		C-4″, C-10″	5.30 m	119.6			5.53 dt	118.7		
3″		142.3				140.5				141.9		
4″	2.13 m	39.5	C-3″, C-5″	C-2″, C-6″	2.81 d (6.5)	42.2	C-3″, C-5″	C-2″, C-6″	2.15 m	35.5		
5″	2.10 m	26.3	C-4″, C-6″	C-3″	5.68 m	128.4			1.75	26.8	C-4″	C-3″
6″	5.10 m	123.6			5.66 d (15)	135.9			4.08 t (6.5)	75.5		
7″		132.0				82.2				147.2		
8″	1.62 s	17.7	C-7″	C-6″, C-9″	1.35 s	24.3	C-7″	C-6″, C-8″	4.95 s, 4.87 t	111.4		C-6″
9″	1.69 s	25.7	C-7″	C-6″, C-8″	1.35 s	24.3	C-7″	C-6″, C-8″	1.76 s	16.7	C-7″	C-6″, C-8″
10″	1.74 s	16.7	C-3″	C-2″, C-4″	1.74 s	16.8	C-3″	C-2″	1.74 s	17.6	C-3″	C-2″, C-4″
4-OH	5.32 bs											
6-OH	14.02 s		C-1	C-2, C-6	14.00 s		C-6	C-1, C-5	13.99 s			C-1, C5

RESULTS AND DISCUSSION

Compound **1** was isolated as a pale yellow oil from the *n*-hexane extract of *H. olympicum* L. cf. *uniflorum*. The HR-ESIMS indicated an $[M - H]^-$ ion at *m/z* 345, suggesting a molecular formula of $C_{21}H_{30}O_4$. The ^{1}H and ^{13}C NMR data (Table 1) were indicative of an acylphloroglucinol with a terpene substituent. The ^{1}H NMR spectrum showed two signals for hydroxy groups, one of which was highly deshielded and hydrogen-bonded (δ 14.02) and the other of which appeared as a broad singlet at δ 5.32. Other signals observed in the ^{1}H NMR spectrum included two *meta*-coupled aromatic protons (δ 5.98 d, *J* = 2.5 Hz; 5.92 d, *J* = 2.5 Hz), two olefinic protons (δ 5.51 m; 5.10 m), one methine (δ 3.66 m), four methylene groups, three methyl singlets (δ 1.74, 1.69, 1.62), one methyl doublet (δ 1.12, *J* = 6.5 Hz), and one methyl triplet (δ 0.89, *J* = 7.5 Hz). The ^{13}C NMR spectrum displayed signals for six aromatic carbons, three of which were highly deshielded, implying that these carbons were attached to electron-withdrawing groups. The pattern of these signals suggested a 1,3,5-trihydroxybenzene (phloroglucinol) structure. In the HMBC spectrum the hydrogen-bonded proton showed a ^{2}J correlation with the carbon to which it was directly attached (δ 167.5, C-6) and ^{3}J correlations with an aromatic methine carbon (δ 96.5, C-5) and a quaternary aromatic carbon (δ 105.0, C-1), confirming the position of the hydroxy group. The other aromatic hydrogen at δ 5.92 was then placed at C-3, as it was *meta*-coupled to H-5. This was further confirmed by HMBC correlations between this proton and C-1, C-5, and a deshielded carbon (δ 161.9, C-4). The second hydroxy group was therefore placed between the aromatic protons at C-4.

The substituent at C-1 included a methine multiplet (δ 3.66, H-2′), a methylene multiplet (δ 1.37, 1.80, H_2-3′), a methyl triplet (δ 0.89, H_3-4′), and a methyl doublet (δ 1.12, H_3-5′). In the COSY spectrum, the methylene (H_2-3′) was coupled to the methyl triplet (H_3-4′), and the methine multiplet (H-2′) was coupled to the methyl doublet (H_3-5′). The HMBC spectrum revealed cross-peaks between this methyl doublet and C-2′, C-3′, and a carbonyl carbon (δ 210.4, C-1′), which could interact with the hydroxy group at C-6 via a hydrogen bond and be responsible for the downfield resonance of this hydroxy group. This confirmed the 2-methylbutanoyl side-chain at C-1.

The final substituent at C-2 included an oxymethylene group (δ 4.57 d, *J* = 6.5 Hz, C-1″), which showed long-range $^{1}H-^{13}C$ correlations with a deshielded aromatic carbon (δ 162.6, C-2) and two carbons associated with an olefinic group (δ 118.2, C-2″; δ 142.3, C-3″). In the HMBC spectrum, an olefinic proton (δ 5.51 m) at C-2″ was coupled to the carbon of a methyl moiety (δ 16.7, C-10″) and to a methylene carbon (δ 39.5, C-4″) via three bonds. This methylene group (δ 2.13 m, H-4″) showed ^{2}J correlations with C-3″ and C-5″ (δ 26.3) and ^{3}J correlations with C-2″ and C-6″ (δ 123.6). Two methyl singlets (δ 1.62, 1.69) were coupled to C-6″ and a quaternary carbon associated with this olefinic carbon (δ 132.0, C-7″), therefore completing the substituent at C-2. This side-chain consisted of 10 carbons, including two olefinic and three methyl groups, and was characteristic of a geranyl group. The COSY and NOESY spectra also

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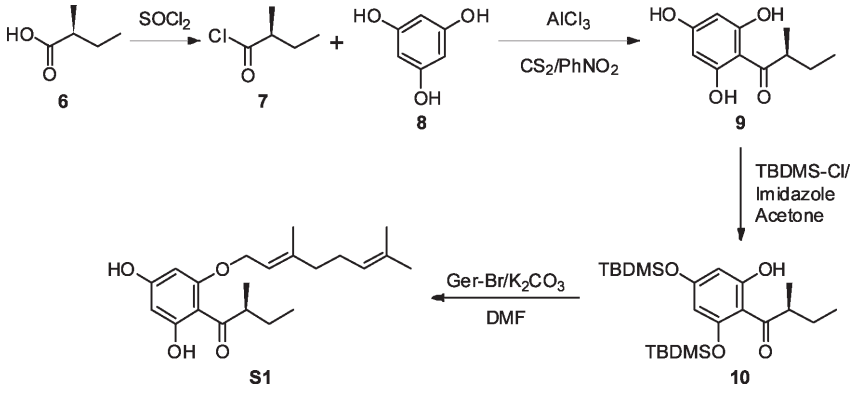

Figure 1. Synthesis of compound **S1**.

provided evidence for the geranyl side-chain. Both double bonds were assigned the *trans*-configuration on the basis of the nature of the biosynthesis of a geranyl side-chain from two isoprene units. This was also supported by an NOE correlation between H-2″ and H_2-4″. The ^{1}H and ^{13}C NMR data on the geranyloxy side-chain showed good agreement with reported data.[7]

The position of the *O*-geranyl substituent was assigned as *ortho* to the 2-methylbutanoyl group on the basis of the HMBC correlations as described and also the observation that the *meta*-coupled aromatic protons were nonequivalent. The configuration at the C-2′ stereogenic center can be identified by comparing the specific rotation of this compound with that of the synthetic compound **S1**, which was synthesized from (+)-(*S*)-2-methylbutanoic acid (Figure 1). The specific rotations for **1** and **S1** were found to be $[\alpha]_D^{22}$ +6.0 (*c* 0.25, $CHCl_3$) and $[\alpha]_D^{22}$ +6.0 (*c* 0.30, $CHCl_3$), respectively. The data implied that the stereogenic center in the natural product **1** was of the *S*-configuration. Compound **1** was therefore identified as the new natural product (*S*)-4,6-dihydroxy-2-*O*-(3″,7″-dimethyl-2″,6″-octadienyl)-1-(2′-methylbutanoyl)benzene and given the trivial name olympicin A. A patent was filed for this compound for its application as a potential antibacterial agent.[8]

Compound **2** was isolated as a pale yellow oil from the CH_2Cl_2 extract of *H. olympicum* L. cf. *uniflorum*. High-resolution ESIMS gave an $[M + H]^+$ ion at *m/z* 363, indicating a molecular formula of $C_{21}H_{30}O_5$. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) were similar to those of compound **1**. The ^{1}H NMR signals indicated the presence of a 2-methylbutanoyl phloroglucinol nucleus with one highly deshielded hydrogen-bonded hydroxy group (δ 14.00), two *meta*-coupled aromatic protons (δ 5.90 d, *J* = 2 Hz, H-3; 5.99 d, *J* = 2 Hz, H-5), one methine multiplet (δ 3.63, H-2′), one methylene (δ 1.37 m, 1.80 m, H_2-3′), one methyl triplet (δ 0.88, *J* = 7.5 Hz, H_3-4′), and one methyl doublet (δ 1.12, *J* = 7.5 Hz, H_3-5′). The ^{13}C NMR signals were again indicative of a phloroglucinol moiety with six aromatic carbons, comprising three deshielded quaternary carbons (δ 167.5, C-6; 162.4, C-2; 162.1, C-4), one quaternary carbon at δ 105.9 (C-1), and two methines at δ 96.6 (C-5) and 91.7 (C-3). The presence of a carbonyl carbon (δ 210.3, C-1′), one methine (δ 46.1, C-2′), one methylene (δ 26.9, C-3′), and two methyl carbon signals (δ 11.9, C-4′; 16.7, C-5′) again suggested a 2-methylbutanoyl side-chain in **2**. The structure of the 2-methylbutanoyl-phloroglucinol nucleus was further confirmed by HMBC correlations, which were described earlier in the structural elucidation of compound **1**.

The remaining signals in the ^{1}H NMR spectrum included three olefinic protons (δ 5.30 m, H-2″; 5.68 m, H-5″; 5.66 d, *J* = 15 Hz, H-6″), two methylene doublets including one shifted downfield at δ_H 4.58 (*J* = 6.5 Hz, H_2-1″) and one at δ 2.81 (*J* = 6.5 Hz, H_2-4″), two equivalent methyl groups integrating to six protons (δ 1.35 s, H_3-8″, H_3-9″), and an additional methyl singlet (δ 1.74, H_3-10″). The remaining signals in the ^{13}C NMR spectra included one quaternary olefinic carbon (δ 140.5, C-3″), three olefinic methines (δ 119.6, C-2″; 128.4, C-5″; 135.9, C-6″), an oxygenated quaternary carbon (δ 82.2, C-7″), two methylenes (δ 65.6, C-1″; 42.2, C-4″), two equivalent methyl carbons at δ 24.3 (C-8″, C-9″), and a further methyl carbon at δ 16.8 (C-10′). An oxymethylene (δ 4.58 d, H_2-1″) was coupled to the olefinic proton at δ 5.30 (H-2″) in the COSY spectrum. It also displayed ^{1}H−^{13}C ^2J correlation to the olefinic carbon at δ 119.6 (C-2″) and ^3J correlation to a quaternary olefinic carbon (δ 140.5, C-3″) to which a methyl group (δ 1.74 s, H_3-10″) was directly attached. The C-10″ methyl group was correlated to a methylene carbon at δ_C 42.2 (C-4″) in the HMBC spectrum, thus placing the methylene group (δ 2.81 d, H_2-4″) next to C-3″. This methylene doublet in turn displayed ^{1}H−^{13}C correlations to four olefinic carbons, confirming its position between the two olefinic bonds. An olefinic multiplet at δ_H 5.68 (H-5″) was placed between the methylene group (H_2-4″) and the olefinic doublet (δ 5.66, H-6″), as they showed correlations in the COSY spectrum. The splitting of the olefinic multiplet (δ 5.68, H-5″) was due to the fact that the signal was split by both the neighboring methylene (H_2-4″) and the C-6″ olefinic proton. The olefinic proton at H-6″ appeared as a clear doublet because the signal was only split by the neighboring H-5″. The large coupling constant between H-5″ and H-6″ (*J* = 15 Hz) indicated that these two protons were in a *trans*-configuration. The equivalent methyl groups (δ 1.35 s, 6 H, H_3-8″, H_3-9″) at the terminus of the side-chain were directly attached to a quaternary carbon (δ 82.2, C-7″), which was shifted downfield by a hydroxy group directly attached to this carbon. This connection was supported by HMBC correlations between the methyl singlet and C-7″ and the olefinic carbon at C-6″. This completed the structural elucidation of compound **2**. The specific rotation of this compound was found to be $[\alpha]_D^{22}$ +5.8 (*c* 0.22, $CHCl_3$), which might again imply that C-2′ was of an *S*-configuration (synthetic compound **S1**, $[\alpha]_D^{22}$ +6.0 (*c* 0.30, $CHCl_3$)). Compound **2** was therefore identified as 4,6-dihydroxy-2-*O*-(7″-hydroxy-3″,7″-dimethyl-2″,5″-octadienyl)-1-(2′-methylbutanoyl)-benzene, assigned as olympicin B, and is described here for the first time.

Compound **3** was isolated as a pale yellow oil from the CH_2Cl_2 extract of *H. olympicum*. The HR-ESIMS gave an $[M - H]^-$ ion

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Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data and $^{1}H-^{13}C$ Long-Range Correlations of **4** and **5** Recorded in $CDCl_3$

	4				5				
position	^{1}H (*J*, Hz)	^{13}C	^{2}J	^{3}J	position	^{1}H (*J*, Hz)	^{13}C	^{2}J	^{3}J
1		105.9			1		105.5		
2		162.4			2		162.3		
3	5.91 d (2.5)	91.7		C-1, C-5	3	6.02 d (2.5)	92.7		C-1, C-5
4		162.0			4		162.8		
5	5.98 d (2.5)	96.6	C-6	C-1, C-3	5	5.98 d (2.5)	96.8		C-3
6		167.5			6		167.6		
1′		210.3			1′		210.0		
2′	3.63 m	46.1			2′	3.63 m	46.1		
3′	1.35 m, 1.80 m	26.8			3′	1.38 m, 1.81 m	26.5		
4′	0.88 t (7.5)	11.9	C-3′	C-2′	4′	0.90 t (7.5)	12.0	C-3′	C-2′
5′	1.11 d (6)	16.6	C-2′	C-1′, C-3′	5′	1.1 d (7)	16.6	C-2′	C-1′, C-3′
1″	4.58 d (6.5)	65.6	C-2″	C-3″	1″	4.67 m	66.4		
2″	5.52 m	119.2			2″	5.50 m	121.9		
3″		141.2			3″		138.5		
4″	2.21 m	35.2			4″	2.30 m	37.0		
5″	1.75 m	26.8			5″	1.65 m, 1.90 m	26.5		
6″	4.32 t	89.0			6″	2.77 dt	65.1		
7″		143.3			7″		59.1		
8″	5.02 s, 5.05 t	114.6		C-6″, C-9″	8″	1.31 s	18.9	C-7″	C-6″, C-9″
9″	1.75 s	17.2	C-7″	C-6″, C-8″	9″	1.34 s	24.6	C-7″	C-6″, C-8″
10″	1.75 s	16.6	C-3″	C-2″, C-4″	10″	1.76 s	16.0	C-3″	C-2″, C-4″
4-OH	5.32 bs				1-OH	13.95 s			C-1, C-5
6-OH	13.99 s		C-6	C-1, C-5	5-OH	6.90 bs			

at *m/z* 361, suggesting a molecular formula of $C_{21}H_{30}O_5$. The ^{1}H and ^{13}C NMR spectroscopic data (Table 3) were again similar to those of compound **1**. The common signals in the ^{1}H NMR spectrum included one highly deshielded hydrogen-bonded hydroxy group (δ 13.99, 6-OH), two *meta*-coupled aromatic protons (δ 5.92 d, *J* = 2 Hz, H-3; 5.98 d, *J* = 2 Hz, H-5), one methine (δ 3.64 m, H-2′), one methylene (δ 1.37 m, 1.80 m, H_2-3′), one methyl triplet (δ 0.88 t, *J* = 7.5 Hz, H_3-4′), and one methyl doublet (δ 1.12 d, *J* = 6.5 Hz, H_3-5′). In the ^{13}C NMR spectrum, signals suggesting a (2-methylbutanoyl)phloroglucinol nucleus were again seen, including three deshielded quaternary aromatic carbons (δ 167.6, C-6; 162.4, C-2; 161.9, C-4), one quaternary aromatic carbon (δ 105.9, C-1), two methine aromatic carbons (δ 91.6 C-3; 96.6, C-5), one carbonyl carbon (δ 210.3, C-1′), one methine (δ 46.1, C-2′), one methylene (δ 26.8, C-3′), and two methyl carbons (δ 11.9, C-4′; 16.7, C-6′). HMBC correlations further confirmed the structure of the (2-methylbutanoyl)phloroglucinol nucleus and were similar to those described in the structural elucidation of compound **1**.

The ^{1}H NMR signals for the side-chain at the 2-*O* position consisted of four methylenes, including one shifted downfield (δ 4.57 d, *J* = 6.5 Hz, H_2-1″) and an *exo*-methylene (δ 4.87 t, 4.95 s, H_2-8″), one olefinic proton (δ 5.53 dt, H-2″), one deshielded methine triplet (δ 4.08, *J* = 6.5 Hz, H-6″), and two methyl singlets (δ 1.76, H_3-9″; 1.74, H_3-10″). Ten carbons were accountable for this side-chain, again indicative of a geranyl moiety. These included two methines (δ 118.7, C-2″; 75.5, C-6″), four methylenes (δ 65.6, C-1″; 35.5, C-4″; 26.8, C-5″; 111.4, C-8″), two quaternary olefinic carbons (δ 141.0, C-3″; 147.2, C-7″), and two methyl carbons (δ 16.7, C-9″; 17.6, C-10″).

The oxymethylene group showed HMBC correlations with an olefinic carbon at δ 118.7 (C-2″) and COSY correlations to the olefinic proton associated with this carbon (δ 5.53). A methyl singlet at δ 1.74 (H_3-10″) showed HMBC correlations with C-2″, an olefinic carbon to which it was directly attached (δ 141.9, C-3″), and to a methylene carbon at δ 35.5 (C-4″). The methylene protons at C-4″ were coupled to another methylene group (δ 1.75 m), which was coupled to a methine triplet (δ 4.08, *J* = 6.5 Hz, H-6″), as shown in the COSY spectrum. This triplet was shifted downfield and was attached to a carbon at δ 75.5 (C-6″), indicating that a hydroxy group should be attached here, and this was supported by the mass spectrum. The remaining methyl group (δ 1.76) was placed at C-7″, as it exhibited a ^{2}J HMBC correlation to a quaternary carbon at δ 143.3 (C-7″) and ^{3}J correlations to the oxygenated methine carbon (C-6″) and to an olefinic carbon at δ 111.4 (C-8″) bearing two *exo*-methylene protons. The specific rotation of **3** was $[\alpha]_D^{22}$ +2.5 (*c* 0.41, $CHCl_3$). Although it showed the same direction of rotation as **S1**, the configuration of **3** could not be determined, as two stereogenic centers were present in this compound. Compound **3** was therefore identified as the new natural product 4,6-dihydroxy-2-*O*-(6″-hydroxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene and was given the trivial name olympicin C.

Compound **4** was isolated as a pale yellow oil from the CH_2Cl_2 extract of *H. olympicum* L. cf. *uniflorum*. High-resolution ESIMS gave an $[M - H]^-$ ion at *m/z* 377, indicating a molecular formula

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Table 3. MIC (μM) Values of Compound 1 and Control Antibiotics against *Staph. aureus*, *Mycobacterium* species, *P. aeruginosa*, and *Salmonella enterica* serovar Typhimurium

strain	control	control (μM)	compound **1** (μM)
S. aureus			
SA-1199B	norfloxacin	100	2.9
XU212	tetracycline	288	2.9
RN4220	erythromycin	349	2.9
ATCC25923	norfloxacin	3.1	2.9
EMRSA-15	oxacillin	79	1.45
EMRSA-16	oxacillin	1276	2.9
Mycobacterium			
M. smegmatis ATCC 14468	isoniazid; ethambutol	14.6; 2.4	11.6
M. fortuitum ATCC 6841	isoniazid; ethambutol	29.2; 2.4	23.2
M. smegmatis MC^2 2700	isoniazid; ethambutol	14.6; 2.4	11.6
M. phlei ATCC 11758	isoniazid; ethambutol	14.6; 2.4	11.6
P. aeruginosa			
K 1119	norfloxacin	6.2	>1 mM
K 767	norfloxacin	6.2	>1 mM
S. enterica serovar Typhimurium			
L 354	tetracycline	2.3	>1 mM
L 10	tetracycline	2.3	>1 mM

of $C_{21}H_{30}O_6$. The ^{1}H and ^{13}C NMR data (Table 2) for compound **4** were similar to those of compound **3**. The characteristic ^{1}H NMR features of a (2-methylbutanoyl)phloroglucinol were again seen in this compound, including one highly deshielded hydrogen-bonded hydroxy group (δ 13.99, 6-OH), two *meta*-coupled aromatic protons (δ 5.91 d, J = 2.5 Hz, H-3; 5.98 d, J = 2.5 Hz, H-5), one methine (δ 3.63 m, H-2′), one methylene (δ 1.35 m, 1.80 m, H$_2$-3′), one methyl triplet (δ 0.88, J = 7.5 Hz, H$_3$-4′), and one methyl doublet (δ 1.11, J = 6 Hz, H$_3$-5′). Signals in the ^{13}C NMR spectrum accounting for a (2-methylbutanoyl)phloroglucinol nucleus consisted of three deshielded quaternary aromatic carbons (δ 167.5, C-6; 162.3, C-3; 162.0, C-4), one quaternary aromatic carbon (δ 105.9, C-1), two aromatic methine carbons (δ 91.7, C-3; 96.6, C-5), one carbonyl carbon (δ 210.3, C-1′), one methine (δ 46.1, C-2′), one methylene (δ 26.8, C-3′), and two methyl carbons (δ 11.9, C-4′; 16.6, C-5′). The connection of these protons and carbons was made through HMBC studies and was identical to those seen in the structural elucidation of compound **1**.

The ^{1}H NMR signals for the side-chain at the 2-*O* position consisted of four methylenes, including an oxymethylene (δ 4.58 d, J = 6.5 Hz, H$_2$-1″) and an *exo*-methylene (δ 5.02 s, 5.05 t, H$_2$-8″), one olefinic proton (δ 5.52 dt, H-2″), one deshielded methine triplet (δ 4.32, J = 6.5 Hz, H-6″), and two overlapping methyl singlets (δ 1.75, 6H, H$_3$-9″, H$_3$-10″). Ten carbon signals were observed in the ^{13}C NMR spectrum for this side-chain, including two methines (δ 89.0, C-6″; 119.2, C-2″), four methylenes (δ 65.6, C-1″; 35.2, C-4″; 26.8, C-5″; 114.6, C-8″), two quaternary olefinic carbons (δ 141.2, C-3″; 143.3, C-7″), and two methyl carbons (δ 17.2, C-9″; 16.6 C-10″). These signals were indicative of a geranyl derivative and were again similar to those of compound **3**.

The oxymethylene group (δ 4.58, H$_2$-1″) exhibited HMBC correlations with two carbons at δ_C 119.2 (C-2″) and 141.2 (C-3″) and COSY correlation with the olefinic proton at C-2″. The methyl singlet at δ 1.74 (H-10″) showed a ^2J HMBC correlation to the olefinic carbon to which it was directly attached (C-3″) and ^3J HMBC correlations to C-2″ and a methylene carbon at δ 35.2 (C-4″). The methylene protons at C-4″ (2.21 m) were coupled to another methylene group (δ 1.75 m, H-5″), which was coupled to a methine triplet (δ 4.32, H-6″), as shown in the COSY spectrum. This triplet was shifted downfield and was attached to a carbon at δ 89.0 (C-6″), indicating that an electron-withdrawing group with a stronger deshielding effect than a hydroxy group was present at this carbon (δ_C 75.5 for hydroxy-bearing C-6″ in compound **3**). This chemical shift was characteristic of a hydroperoxy-bearing methine. The molecular formula for this compound ($C_{21}H_{30}O_6$) indicated that it had an extra oxygen when compared to that of compound **3** ($C_{21}H_{30}O_5$). The remaining methyl group (δ_H 1.75, H-9″) was placed at C-7″ (δ_C 143.3), as the methyl singlet showed HMBC correlations to this carbon, the hydroperoxy-bearing carbon (C-6″), and the *exo*-methylene carbon at δ_C 114.6 (C-8″). This was further confirmed by HMBC correlations between the *exo*-methylene group and C-6″ and C-9″. This completed the structural elucidation of compound **4**. Like **S1** and other acylphloroglucinols isolated from *H. olympicum* L. cf. *uniflorum*, the specific rotation of compound **4** showed a positive value ($[\alpha]_D^{22}$ +2.7 (c 0.32, $CHCl_3$)). However, two stereogenic centers (C-2′ and C-6″) were present in **4**, and thus the configuration at each stereogenic center could not be determined by direct comparison with the specific rotation of **S1**. A further experiment using a starch-iodide test strip (Fisher) was conducted to confirm the presence of the hydroperoxide group in this compound. A starch-iodide test strip was dipped into a $CHCl_3$ solution of compound **4** (approximately 2 mg/mL) and showed a positive color reaction (light brown). $CHCl_3$ was used as a negative control and did not result in any color change. A $CHCl_3$ solution of mCPBA (20 mg/mL) was used as a positive control, and it turned the test strip dark blue. When a lower concentration of mCPBA was used (approximately 2 mg/mL), the test strip turned light brown, implying that the color change was dependent on the concentration of the hydroperoxide present. Compound **4** was therefore identified as 4,6-dihydroxy-2-*O*-(6″-hydroperoxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene (olympicin D) and is reported here for the first time.

Compound **5** was isolated as a pale yellow oil from the CH_2Cl_2 extract of *H. olympicum* L. cf. *uniflorum*. The molecular formula of compound **5** was $C_{21}H_{30}O_5$, indicated by an $[M + H]^+$ ion at m/z 363 in the high-resolution ESIMS. The ^{1}H NMR spectrum (Table 2) revealed signals typical of a (2-methylbutanoyl)phloroglucinol, including a broad signal accounting for a hydroxy group (δ 6.90, 4-OH), two *meta*-coupled aromatic protons (δ 6.02 d, J = 2.5, H-3; 5.98 d, J = 2.5, H-5), a methine multiplet (δ 3.63, 1H, H-2′), and a methylene group (δ 1.38 m, 1.81 m, H$_2$-3′). More interestingly, duplication of the following signals was observed: a highly deshielded hydrogen-bonded singlet (δ 13.95, 6-OH), a methyl triplet (δ 0.90, J = 7.5, H$_3$-4′), and a methyl doublet (δ 1.13, J = 7, H$_3$-5′). The carbon signals corresponding to the phloroglucinol nucleus included three deshielded aromatic carbons (δ 167.6, C-6; 162.3, C-2; 162.8, C-4), a quaternary aromatic carbon (δ 105.5, C-1), and two aromatic methines (δ 92.7, C-3; 96.8, C-5). Duplication of the signals in the ^{13}C NMR spectrum was also observed for the 2-methylbutanoyl side-chain: a carbonyl carbon (δ 210.1 and 210.0, C-1′), a methine

(δ 46.09 and 46.12, C-2′), a methylene (δ 26.93 and 26.96, C-3′), and two methyl carbons (δ 11.96 and 12.00, C-4′; 16.52 and 16.55, C-5′). The HMBC correlations observed for the acylphloroglucinol nucleus of this compound were identical to those of compound **1** and were as described previously. The duplication of NMR signals was indicative of the presence of two rotamers, and this phenomenon has been observed in some natural products, including acylphloroglucinols.[9] In the case of acylphloroglucinols, two isomers in rotameric forms were possible: one with the acyl chain above the plane of the phloroglucinol and the other below the plane. Molecular modeling calculation was carried out for this compound. Since two relatively stable conformations were possible, two different energy minima were expected. However, there was only one energy minimum observed in the calculation.

The ^{1}H NMR signals for the side-chain at the 2-*O* position included three methylenes (δ 4.67 m, H_2-1″; 2.30 m, H_2-4″; 1.65 m, 1.90 m, H_2-5″), one olefinic proton (δ 5.50 m, H-2″), one deshielded oxymethine (δ 2.77 dt, H-6″), and three methyl singlets (δ 1.34 s, H_3-8″; 1.31 s, H_3-9″; 1.76 s, H_3-10″). Ten carbon signals were observed in the ^{13}C NMR spectrum for this side-chain, including three methylenes (δ 66.4, C-1″; 37.0, C-4″; 26.5, C-5″), an olefinic carbon (δ 121.9, C-2″), an oxymethine (δ 65.1, C-6″), and three methyl carbons (δ 24.6, C-8″; 18.9, C-9″; 16.0, C10″).

Due to the small quantity of the compound, signals in the HMBC spectrum were very weak. The COSY spectrum and the structures of the other related derivatives isolated from this plant played an important role in the structural elucidation of this compound. As with the other acylphloroglucinols, the oxymethylene group (δ 4.67 m, H_2-1″) was placed at C-2 of the acylphloroglucinol. The oxymethylene protons showed a COSY correlation with the olefinic proton (δ 5.50 m, H-2″), thus placing the olefinic proton at C-2″. The methyl group shifted downfield at δ_H 1.76 (H_3-10″) showed a ^2J HMBC correlation to a quaternary olefinic carbon at δ 138.5 (C-3″) and ^3J HMBC correlations to C-2″ and a methylene carbon at δ 37.0 (C-4″). The methylene protons at C-4″ (δ 2.30 m) showed a COSY correlation to a further methylene group (δ 1.65 m, 1.90 m, C-5″), which was coupled to an oxymethine, as revealed by the COSY spectrum (δ 2.77 dt, H-6″). The remaining methyl groups at δ 1.34 (H_3-8″) and 1.31 (H_3-9″) showed HMBC correlations to δ 18.9 (C-9″) and 24.6 (C-8″), respectively, indicating that the methyl groups were geminal to each other. These methyl groups also correlated to a quaternary carbon at δ 59.1 (C-7″) and the oxymethine carbon (C-6″). The ^{13}C NMR chemical shifts of C-6″ and C-7″ were indicative of an epoxy moiety,[10] and the ^{1}H and ^{13}C NMR data of this side-chain showed close agreement with the corresponding substituent of 6′,7′-epoxygeranyloxypsoralen.[10] Again, this compound showed a positive specific rotation ($[\alpha]_D^{22}$ +2.6 (*c* 0.19, $CHCl_3$)). However, due to the presence of two stereogenic centers (C-2′ and C-6″) in the molecule, the configuration at the stereocenter could not be determined by direct comparison with that of S1. Compound **5** was therefore identified as the new 4,6-dihydroxy-2-*O*-(6″,7″-epoxy-3″,7″-dimethyloct-2″-enyl)-1-(2′-methylbutanoyl)benzene and was given the trivial name olympicin E.

Olympicin A (**1**) displayed exceptional activity against all of the *Staph. aureus* strains tested, with MIC values ranging from 0.5 to 1 mg/L (Table 3). It was more active than the control antibiotics against the MDR and epidemic strains and as active as norfloxacin with the standard susceptibility testing strain (ATCC 25923). The activity of olympicin A seemed to be unaffected by the MDR mechanisms, as shown by the fairly consistent MIC values against the different effluxing strains. It was also active against four *Mycobacterium* strains at MIC values ranging from 4 to 8 mg/L, but was not as active as the control antibiotics. Olympicin A was not active against any of the Gram-negative species at 512 mg/L. This may result from the impermeability of the outer membrane of the Gram-negative bacteria, which prevents the influx of chemicals from the surrounding environment into the cells, or may be due to the different efflux pumps these bacteria express.

Compounds **2**−**5** (olympicins B−E) were moderately active against the *Staph. aureus* strains, with MICs ranging from 64 to 128 mg/L. The simple nature of olympicin A and the ease of its synthesis, coupled with its activity toward Gram-positive strains of *Staph. aureus*, some of which are effluxing and methicillin-resistant, make this chemotype worthy of further evaluation, and synthetic efforts are underway to optimize its antibacterial action. The need for new topical antibacterials, particularly those used in the decolonization of MRSA from patients, offers a potential use for these simple acylphloroglucinols, particularly given the resistance occurring to conventional agents such as mupirocin and fusidic acid.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Bellingham and Stanley ADP 200 polarimeter. UV spectra were recorded on a Thermo Electron Corporation Helios spectrophotometer, and IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer. Chemical shift values (δ) are reported in ppm, relative to appropriate internal solvent standard, and *J* values are given in Hz. Mass spectra were recorded on a Finnigan MAT 95 high-resolution, double-focusing, magnetic sector mass spectrometer. Accurate mass measurement was achieved using voltage scanning of the accelerating voltage. This was nominally 5 kV, and an internal reference of heptacosa was used. Resolution was set between 5000 and 10 000.

Plant Material. The aerial parts of *H. olympicum* L. cf. *uniflorum* N. Robson (accession number 1969-31184) were collected from the Royal Botanic Garden at Wakehurst Place, Surrey, which forms part of the National *Hypericum* Collection.

Extraction and Isolation. Dried, powdered plant material (937 g) was sequentially extracted with 3.5 L of *n*-hexane, CH_2Cl_2, and MeOH using a Soxhlet apparatus. The *n*-hexane and CH_2Cl_2 extracts were active at a concentration of 32 and 16 mg/L, respectively, whereas the MeOH extract was active at 512 mg/L. The *n*-hexane extract (15.2 g) was fractionated by VLC (silica gel $PF_{254+366}$; Merck) using a step-gradient solvent system from 100% *n*-hexane to 100% EtOAc with a 10% increment and a final MeOH wash. VLC fractions 6 to 8 were active against SA-1199B with an MIC value of 64 mg/L. They displayed similar TLC profiles and were combined (total of 842.0 mg). The combined fraction was separated by Sephadex LH-20 (Amersham Biosciences) chromatography, giving five fractions eluted with $CHCl_3$/MeOH (1:1) and one fraction eluted with MeOH. The fraction eluted with MeOH (80.9 mg) was active at an MIC of 1 mg/L. Compound **1** (29.1 mg) was isolated from this fraction using preparative TLC (silica; toluene/EtOAc/HOAc, 80:18:2, R_f = 0.62, yield 0.0031%).

A portion (2.5 g) of the CH_2Cl_2 extract was applied to a Sephadex LH-20 column, giving five fractions eluted with $CHCl_3$/MeOH (1:1) and one fraction eluted with MeOH. Fractions 4 to 6 exhibited excellent antibacterial activity at 0.5 mg/L. They showed similar TLC profiles and

were combined, giving 62.0 mg. The combined fraction was directly purified by p-TLC (silica; toluene/EtOAc/HOAc, 75:23:2), yielding compounds **2** (4.2 mg, R_f = 0.48, yield 0.00045%), **3** (3.8 mg, R_f = 0.44, yield 0.00041%), and **4** (1.8 mg, R_f = 0.34, yield 0.00019%). Sephadex fraction 3 (140.8 mg) was active at 512 mg/L. It was further separated by SPE on a silica gel column using a step-gradient system from 100% *n*-hexane to 100% EtOAc. SPE fraction 8 (17.8 mg), which was eluted with 70% EtOAc in *n*-hexane, was purified by p-TLC (silica; toluene/EtOAc/HOAc, 75:23:2), yielding compound **5** (1.3 mg, R_f = 0.57, yield 0.00013%). All compounds gave an orange color reaction with vanillin-H_2SO_4 spray on a TLC plate.

*4,6-Dihydroxy-2-O-(3″,7″-dimethyl-2″,6″-octadienyl)-1-(2′-methylbutanoyl)benzene (**1**), olympicin A:* pale yellow oil; $[\alpha]_D^{22}$ +6.0 (*c* 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.05), 289 (4.35) nm; IR ν_{max} (thin film) cm^{-1} 3348, 2968, 2931, 1624, 1593, 1448, 1377, 1216, 1162, 1099, 826; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; HR-ESIMS (*m*/*z*) 345.2056 $[M - H]^-$ (calcd for $C_{21}H_{30}O_4$, 345.2071).

*4,6-Dihydroxy-2-O-(7″-hydroxy-3″,7″-dimethyl-2″,5″-octadienyl)-1-(2′-methylbutanoyl)benzene (**2**), olympicin B:* pale yellow oil; $[\alpha]_D^{22}$ +5.8 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.26), 290 (3.62) nm; IR ν_{max} (thin film) cm^{-1} 3338, 2967, 2934, 2873, 1620, 1594, 1448, 1217; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; HR-ESIMS (*m*/*z*) 363.2168 $[M + H]^+$ (calcd for $C_{21}H_{30}O_5$, 363.2172).

*4,6-Dihydroxy-2-O-(6″-hydroxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene (**3**), olympicin C:* pale yellow oil; $[\alpha]_D^{22}$ +2.5 (*c* 0.41, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.44), 288 (4.90) nm; IR ν_{max} (thin film) cm^{-1} 3357, 1734, 1653, 1558, 1540, 1506, 1457; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; HR-ESIMS (*m*/*z*) 363.2163 $[M + H]^+$ (calcd for $C_{21}H_{30}O_5$, 363.2172).

*4,6-Dihydroxy-2-O-(6″-hydroperoxy-3″,7″-dimethyl-2″,7″-octadienyl)-1-(2′-methylbutanoyl)benzene (**4**), olympicin D:* pale yellow oil; $[\alpha]_D^{22}$ +2.7 (*c* 0.32, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.05), 289 (4.35) nm; IR ν_{max} (thin film) cm^{-1} 3397, 2970, 2928, 1622, 1594, 1456, 1217; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 2; HR-ESIMS (*m*/*z*) 379.2120 $[M + H]^+$ (calcd for $C_{21}H_{30}O_6$, 379.2121).

*4,6-Dihydroxy-2-O-(6″,7″-epoxy-3″,7″-dimethyloct-2″-enyl)-1-(2′-methylbutanoyl)benzene (**5**), olympicin E:* pale yellow oil; $[\alpha]_D^{22}$ +2.6 (*c* 0.19, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.05), 289 (3.68) nm; IR ν_{max} (thin film) cm^{-1} 3376, 2970, 2934, 1622, 1593, 1447, 1217; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 2; HR-ESIMS (*m*/*z*) 363.2181 $[M + H]^+$ (calcd for $C_{21}H_{30}O_5$, 363.2166).

Synthesis of Compound 1. *(S)-2-Methylbutanoyl Chloride (**7**) (ref* 11*).* (*S*)-2-Methylbutanoic acid (**6**; 10 g, 97.91 mmol) and $SOCl_3$ (10.71 mL, 146.9 mmol, 1.5 equiv) were heated together at 80 °C under reflux for 2 h. Distillation of the reaction mixture afforded (*S*)-2-methylbutanoyl chloride (10.63 g, 88.21 mmol, 90%): colorless liquid; $[\alpha]_D^{22}$ +10.1 (*c* 0.54, $CHCl_3$); bp 119−120 °C; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Supporting Information.

*(S)-2-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**9**) (ref* 12*).* $AlCl_3$ (46.43 g, 351.8 mmol, 4.1 equiv) was added to a stirred suspension of phloroglucinol (**8**; 10.81 g, 85.8 mmol, 1 equiv) in CS_2 (50 mL). Nitrobenzene (40 mL) was added to the solution over 30 min. The solution was heated under reflux at 55 °C for 30 min. A solution of **7** (10.34 g, 85.8 mmol) dissolved in 5 mL of nitrobenzene was added to the reaction mixture over 30 min, followed by heating for another 30 min. The reaction mixture was allowed to cool with stirring and then poured into an ice−water bath (400 mL). Then 100 mL of 3 M HCl was added. The organic solvents were removed under reduced pressure, and the oily residue containing the acylphloroglucinol was extracted into Et_2O. After the removal of the Et_2O, the crude product was purified by VLC using silica. The fraction eluting with 6:4 *n*-hexane/EtOAc was identified to be the title compound, isolated as a pale yellow oil (9.71 g, 46.19 mmol, 54%): $[\alpha]_D^{22}$ +8.5 (*c* 0.35, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (4.17), 290 (3.97) nm; IR ν_{max} (thin film) cm^{-1} 3297, 1628, 1602, 1222; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Supporting Information; HR-ESIMS (*m*/*z*) 209.0813 $[M - H]^-$ (calcd for $C_{11}H_{14}O_4$, 209.0814).

*(S)-1-(2,4-Bis[(tert-butyldimethylsilyl)oxy]-6-hydroxyphenyl)-2-methylbutan-1-one (**10**) (ref* 13*).* Acylphloroglucinol **9** (9.71 g, 46.19 mmol) was dissolved in 150 mL of dry acetone. Imidazole (3.43 g, 138.6 mmol, 3 equiv) was added to the solution, and the reaction mixture stirred for 5 min before the addition of TBDMS-Cl (14.61 g, 97.0 mmol, 2.1 equiv). The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with $CHCl_3$ and washed with 1 M HCl (150 mL). The solvent was removed under reduced pressure, and the crude product purified by VLC over silica gel to afford TBDMS-protected phloroglucinol **10** in the fraction eluted with 9:1 *n*-hexane/EtOAc. The title compound was isolated as a pale yellow oil (16.4 g, 37.26 mmol, 81%): $[\alpha]_D^{22}$ +4.9 (*c* 0.39, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (4.26), 290 (4.23) nm; IR ν_{max} (thin film) cm^{-1} 3276, 2973, 1688, 1572, 1531, 1256, 1131, 1072, 850; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Supporting Information; HR-ESIMS (*m*/*z*) 437.2554 $[M - H]^-$ (calcd for $C_{23}H_{42}O_4Si_2$, 437.2549).

*(S)-4,6-Dihydroxy-2-O-(3″,7″-dimethyl-2″,6″-octadienyl)-1-(2′-methylbutanoyl)benzene (**S1**), Olympicin A (ref* 13*).* TBDMS-protected acylphloroglucinol **10** (6.6 g, 15.0 mmol) was dissolved in 100 mL of dry DMF, to which anhydrous K_2CO_3 was added (3.1 g, 22.5 mmol, 1.5 equiv). The mixture was stirred for approximately 5 min before the addition of geranyl bromide (3.43 mL, 18 mmol, 1.2 equiv). The mixture was heated at 80 °C for 3 h with stirring. The reaction mixture was poured over H_2O and extracted with $CHCl_3$. The solvent in the organic layer was removed under reduced pressure. The crude product was purified by chromatography over silica gel by VLC. Compound **S1** was eluted with 9:1 *n*-hexane/EtOAc, and removal of the solvents under reduced pressure yielded the title compound as a pale yellow oil. All spectral data were identical to those of the natural product **1**, and the overall yield was 1.6%.

Antibacterial Assay with *Staphylococcus aureus*. Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich Company Ltd., UK. Cation-adjusted Mueller-Hinton broth was obtained from Oxoid and was adjusted to contain 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively. The *Staph. aureus* strains used in this study included ATCC 25923, SA-1199B, RN4220, XU212, EMRSA-15, and EMRSA-16. ATCC 25923 is a standard laboratory strain sensitive to antibiotics.[14] SA-1199B overexpresses the NorA MDR efflux pump.[15] RN4220 possesses the MsrA macrolide efflux protein.[16] XU212 is a Kuwaiti hospital isolate that is a MRSA strain possessing the TetK tetracycline efflux pump.[14] EMRSA-15[17] and EMRSA-16[18] are epidemic strains in the U.K. *Mycobacterium* species used in this study included the fast-growing species *M. smegmatis* ATCC14468, *M. fortuitum* ATCC6841, and *M. phlei* ATCC11758. All were obtained from the National Collection of Type Cultures (NCTC). *M. smegmatis* $MC^2$2700, which possesses the *M. tuberculosis* fatty acid synthase I gene (Fas 1), was obtained from Zimhony et al.[19] Two wild-type Gram-negative bacteria were used in this study, namely, *Pseudomonas aeruginosa* K767 and *Salmonella typhimurium* L354, and both were obtained from the NCTC.

Staph. aureus, *P. aeruginosa*, and *S. typhimurium* strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37 °C prior to MIC determination. *Mycobacterium* species were cultured on Columbia agar (Oxoid) supplemented with 7% defibrinated horse blood (Oxoid) and were subcultured and incubated for 72 h at 37 °C prior to the assay. An inoculum density of 5×10^5 colony forming units of each bacterial strain was prepared in normal saline (9 g/L) by comparison with a 0.5 MacFarland turbidity standard. The inoculum (125 μL) was added to all wells, and the microtiter plate was incubated at 37 °C for the corresponding incubation time. For MIC determination, 20 μL of a 5 mg/mL methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each of the wells and incubated for 20 min. Bacterial growth was indicated by a color change

from yellow to dark blue. The MIC was recorded as the lowest concentration at which no growth was observed.[14]

■ ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

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