Cytotoxic 5-Alkylresorcinol Metabolites from the Leaves of *Grevillea robusta*

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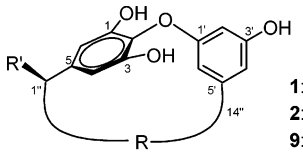
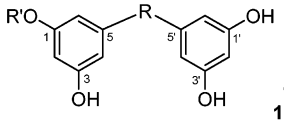
Bioassay-guided fractionation of the MeOH extract of the leaves of *Grevillea robusta* led to the isolation of six new 5-alkylresorcinols, gravicycle (**1**), dehydrogravicycle (**2**), bisgravillol (**3**), dehydrobisgravillol (**4**), dehydrograviphane (**5**), and methyldehydrograviphane (**6**), as well as eight known compounds. The structures of these compounds were determined by spectroscopic and chemical methods. Graviphane (**7**) and methylgraviphane (**8**) were isolated in the pure form for the first time from a natural source. The compounds all showed marginal cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines.

Grevillea robusta A. CUNN (Proteaceae), known commonly as "silky oak", is a tropical ornamental tree native to Australia.[1,2] In Taiwan, it is cultivated as a shade tree. In our continuing search for bioactive molecules from the plants of Taiwan, a MeOH extract of the leaves of *G. robusta* was selected for bioassay-guided fractionation, as it showed significant cytotoxicity against human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines.

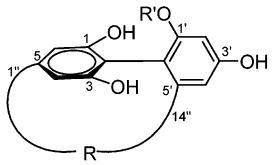
The methanol extract of the leaves of *G. robusta* was suspended in H_2O and partitioned into hexane-, $CHCl_3$-, and EtOAc-soluble fractions. The hexane fraction was chromatographed to afford seven active subfractions, A−G, containing alkylresorcinols with different skeletons and found to be extremely difficult to separate into pure components.[2] In the ^{1}H NMR spectrum of each subfraction, one set of signals between δ 5.8 and 6.5 for aromatic protons and signals between δ 1.0 and 1.7 typical for long chain methylenes were noticed. The spectrum also exhibited olefinic protons near δ 5.3, allylic protons near δ 2.0, and methylene protons adjacent to two double bonds at about δ 2.8, which represent varying degrees of unsaturation in the carbon chain. The integration among the aromatic methines, olefinic methines, and allylic methylenes was not consistent, indicating that each active subfraction was a mixture of resorcinols having the same ring system but with both saturated and unsaturated alkyl substituents. Moreover, mass spectral analysis inferred that the major component in each subfraction was a resorcinol containing a 14-carbon chain having a double bond ($C_{14,1}$). Except the molecular ion peak for $C_{14,1}$, the minor peaks at M + 2, M + 26, and M + 28 were attributable to resorcinols bearing saturated ($C_{14,0}$) and unsaturated ($C_{16,2}$, $C_{16,1}$) chains, respectively. After hydrogenation with Pd/C, each sample exhibited only M + 2 and M + 30 peaks in the mass spectra, corresponding to saturated long chain $C_{14,0}$ and its double homologue $C_{16,0}$. Eventually, by prolonged chromatographic separations on silica gel, small amounts of 14 pure 5-alkylresorcinol metabolites were isolated. Of these, gravicycle (**1**), dehydrogravicycle (**2**), bisgravillol (**3**), dehydrobisgravillol (**4**), dehydrograviphane (**5**), and methyldehydrograviphane (**6**) were new compounds; graviphane (**7**) and methylgraviphane (**8**) were isolated as pure compounds for the first time; and robustol (**9**),[3] dehydrorobustol A (**10**),[3] bis-norstriatol (**11**),[4] 5-[14′-(3″,5″-dihydroxyphenyl)-*cis*-tetradec-6′-en-1-yl]resorcinol (**12**),[3] *cis*-5-*n*-pentadecylresorcinol (**13**),[5] and *cis*-5-*n*-pentadec-8′-enylresorcinol (**14**)[6] were known compounds. The hydrogenated products of **2**, **4**, **5**, **6**, **10**, **12**, and **14** were identical to the saturated analogues of **1**, **3**, **7**, **8**, **9**, **11**, and **13**, respectively. No attempt was made to estimate the amount of each compound in the subfractions. Herein, we describe the isolation, structural elucidation, and cytotoxic properties of these resorcinols.



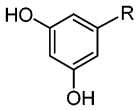
1: R = $(CH_2)_{13}$, R′ = OH
2: R = $(CH_2)_6CH{=}CH(CH_2)_5$, R′ = OH
9: R = $(CH_2)_{13}$, R′ = H
10: R = $(CH_2)_6CH{=}CH(CH_2)_5$, R′ = H



3: R = $(CH_2)_{14}$, R′ = CH_3
4: R = $(CH_2)_7CH{=}CH(CH_2)_5$, R′ = CH_3
11: R = $(CH_2)_{14}$, R′ = H
12: R = $(CH_2)_7CH{=}CH(CH_2)_5$, R′ = H



5: R = $(CH_2)_7CH{=}CH(CH_2)_5$, R′ = H
6: R = $(CH_2)_7CH{=}CH(CH_2)_5$, R′ = CH_3
7: R = $(CH_2)_{14}$, R′ = H
8: R = $(CH_2)_{14}$, R′ = CH_3



13: R = $(CH_2)_{14}CH_3$
14: R = $(CH_2)_7CH{=}CH(CH_2)_5CH_3$

Gravicycle (**1**) was isolated as a white, amorphous powder. The molecular formula was determined to be $C_{26}H_{36}O_5$ from the molecular ion at *m*/*z* 428.2565. From the ^{1}H NMR and COSY spectra, the upfield shifted aromatic signals were assigned as two symmetrical protons [δ 6.46 (s, H-4 and -6)] of a 2,5-disubstituted resorcinol and three *m*-coupled protons [δ 5.83 (t, J = 2.1 Hz, H-6′), 6.26 (t, J = 2.1 Hz, H-4′), and 6.48 (t, J = 2.1 Hz, H-2′)] of a 5′-substituted resorcinol. A 14-carbon long chain containing typical methylenes at δ 1.27 (20H, m, H-3″−12″), 1.45 (2H, m, H-13″), and 1.72 (2H, m, H-2″), benzylic protons at δ 2.34 (2H, t, J = 7.8 Hz, H-14″), and a proton on a benzylic carbon bearing an OH functionality at δ 4.46 (1H, t, J = 6.6 Hz, H-1″) were also observed. The HMBC correlations between H-1″ and C-4, -6 (δ 106.7) and C-5 (δ 143.9) as well as between H-14″ and C-4′ (δ 109.9), C-5′ (δ 146.0), and C-6′ (δ 105.3) indicated that the resorcinols were

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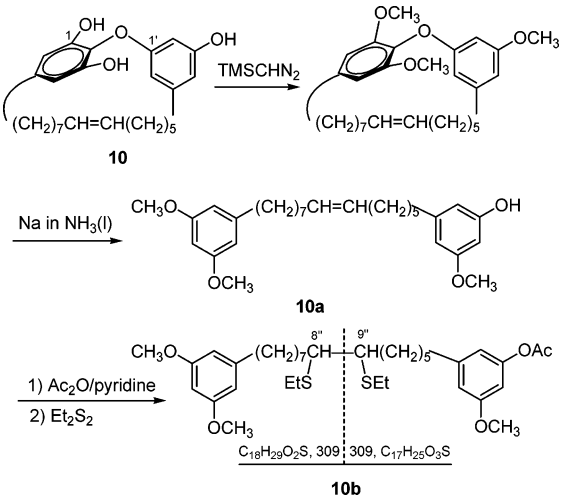
Scheme 1



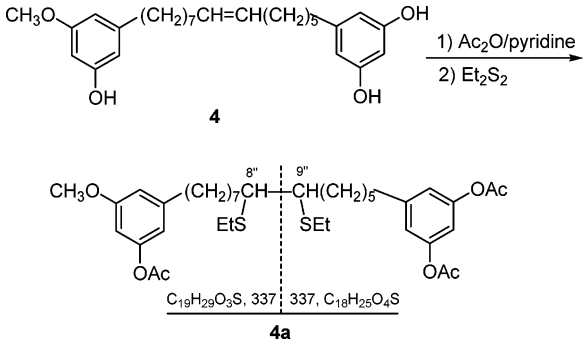
10 **10a** **10b**

attached to both ends of the 1″-hydroxytetradecyl chain. On the basis of the chemical shift of C-2 (δ 130.4), an ether linkage via C-1′ and C-2 between two aromatic rings to form a macrocyclic molecule, as in robustol (**9**), was deduced. This could also be supported by an unusual upfield shift of H-6′ (δ 5.83) compared with H-4′ (δ 6.26) due to the shielding effect by the other non-coplanar aromatic ring. The stereochemistry at C-1″ was determined using a modified Mosher ester method to elucidate the absolute configuration of the secondary alcohol by the application of ^{1}H NMR spectroscopy.[7,8] On the basis of the drawing of a Mosher plane, the positive $\Delta\delta$ for H-2″ (+0.07) as well as the negative $\Delta\delta$ for H-4 (−0.10) and H-6 (−0.01) indicated the *R* configuration at C-1″. Consequently, the structure (*R*)-1″-hydroxyrobustol was assigned for gravicycle (**1**).

Dehydrogravicycle (**2**) was determined to have the molecular formula $C_{26}H_{34}O_5$, two atomic mass units less than that of **1**, by HREIMS. Compounds **2** and **1** exhibited almost identical ^{1}H NMR spectra, except for the appearance of two vinylic protons at δ 5.33 and four allylic protons at δ 2.00 in **2**, indicating the presence of an unsaturation in the long chain. The configuration of the double bond appeared to be *Z*, as evidenced by the allylic carbon resonances at δ 27.6 (C-7″ and -10″) and olefinic carbon resonances at δ 129.8 (C-8″ and -9″).[9] The long chain with a double bond has been proposed as $-(CH_2)_9CH{=}CH(CH_2)_3-$ and/or $-(CH_2)_7CH{=}CH-(CH_2)_5-$ by chemical degradation and biosynthetic consideration.[10,11] The presence of HMBC correlations between benzylic H-1″ (δ 4.46), -14″ (δ 2.35) and methylene carbons at δ 27.9−30.2 instead of the allylic carbon at δ 27.6 ruled out the former possibility. Owing to the small amount of **2**, the position of the double bond in the cyclic structure was determined by chemical transformation using dehydrorobustol A (**10**) as a model (Scheme 1). Methylation of **10** with (trimethylsilyl)diazomethane[12] followed by opening the ring with sodium in liquid ammonia[13] gave **10a**. Its structure was confirmed by the presence of one methoxyl singlet at δ 3.76 (3-OCH_3), two equivalent methoxyl singlets at δ 3.78 (1′- and 3′-OCH_3), and two sets of 1,3,5-trisubstituted benzene ring signals, one set at δ 6.30 (H-2′) and 6.35 (H-4′ and -6′) and the other set at δ 6.23 (H-2), 6.25 (H-6), and 6.32 (H-4). Acylation of **10a** with acetyl chloride and addition of diethyl disulfide afforded **10b**.[14] The EIMS of **10b** ($C_{35}H_{54}O_5S_2$) showed a base peak at *m*/*z* 309, corresponding to either $C_{18}H_{29}O_2S$ or $C_{17}H_{25}O_3S$, indicating the location of a double bond between carbons 8″ and 9″. Comparison of the specific rotation with **1** and the double-bond position with **10** led us to deduce that compound **2** possessed the structure (*R*)-1″-hydroxy-(*Z*)-dehydrorobustol A, and it was named dehydrogravicycle.

Bisgravillol (**3**) was isolated as a white amorphous powder. The HREIMS showed a molecular ion at *m*/*z* 428.2924, consistent with

Scheme 2



4 **4a**

the molecular formula $C_{27}H_{40}O_4$. The ^{1}H NMR and COSY spectra exhibited two slightly different sets of 5-alkylresorcinol signals, one set at δ 6.25 (1H, s, H-2), 6.26 (1H, s, H-4), and 6.33 (1H, s, H-6) and the other at δ 6.17 (1H, s, H-2′) and 6.24 (2H, s, H-4′ and -6′). A methoxyl group at δ 3.76 showed a NOE with H-2 and H-6, indicating its attachment to C-1. Ten methylenes at δ 1.25 for H-3″ to H-12″, two homobenzylic methylenes at δ 1.56 for H-2″ and H-13″, and two benzylic methylenes at δ 2.49 for H-1″ and H-14″ suggested that **3** should be a bis-5-alkylresorcinol derivative with a chain length of 14 carbon atoms. The HMBC correlations of aromatic methines H-4, -6, -4′, and -6′ with the benzylic carbons C-1″ and -14″ (δ 35.8 and 36.0) further confirmed the above structure. Thus, bisgravillol (**3**) was defined as 1-*O*-methyl-5-[14″-(1′,3′-dihydroxyphenyl)tetradec-1″-yl]resorcinol.

Dehydrobisgravillol (**4**) was isolated as a colorless syrup. The HREIMS showed the molecular ion at *m*/*z* 426.2773 for $C_{27}H_{38}O_4$, two hydrogen atoms less than that of **3**. The NMR data of these two compounds showed that they were closely related, but indicated the presence of a $C_{14,1}$ alkenyl chain in **4**. The existence of a vinylic proton signal at δ 5.35 (2H, m) and allylic proton signals at δ 2.02 (4H, m) in the ^{1}H NMR spectrum indicated that **4** was simply an unsaturated analogue of **3**. The *Z* geometry of the double bond was evidenced by the signals of allylic carbons at δ 27.1 and olefinic carbons at δ 129.8. Finally, the position of the double bond was established by the treatment of **4** with acetyl chloride followed by the addition of diethyl disulfide to give **4a** (Scheme 2). The EIMS of **4a** ($C_{37}H_{54}O_7S_2$) showed a base peak at *m*/*z* 337, corresponding to either $C_{19}H_{29}O_3S$ or $C_{18}H_{25}O_4S$. This clearly inferred that the double bond was between carbons 8″ and 9″ in the 14-carbon chain. Therefore, the structure of dehydrobisgravillol (**4**) was (*Z*)-1-*O*-methyl-5-[14″-(1′,3′-dihydroxyphenyl)tetradec-8″-en-1″-yl]resorcinol.

Dehydrograviphane (**5**) showed its molecular ion at *m*/*z* 410.2455 ($C_{26}H_{34}O_4$). Signals assignable to a 14-carbon chain with a double bond were observed at δ 1.25 (12H, m, 6 × CH_2), 1.36 (2H, m, H-13″), 1.58 (2H, m, H-2″), 1.95 (4H, m, 2 × allylic CH_2), 2.26 (2H, t, J = 7.8 Hz, H-14″), 2.59 (2H, t, J = 6.3 Hz, H-1″), and 5.32 (2H, m, 2 vinyl protons). In the aromatic region, a two-proton singlet at δ 6.47 for H-4 and H-6 and two singlets at δ 6.45 and 6.48 for H-2′ and -4′, respectively, confirmed the 2,5- and 5′,6′-disubstituted resorcinol moieties. HMBC connectivities of H-4 and H-6 with C-1″ (δ 36.0) and only H-4′ with C-14″ (δ 34.2) indicated that the terminals of the long chain were attached to C-5 and C-5′ of two resorcinol units. The upfield chemical shifts of quaternary carbons C-2 (δ 103.4) and C-6′ (δ 105.7) suggested a carbon−carbon linkage between two resorcinol rings through C-2 and C-6′ to form a non-coplanar biphenyl skeleton. No optical activity ($[\alpha]_D$ = 0) for **5** proved the presence of a conformation with a plane of symmetry. Four hydroxyl signals at δ 4.73 (2H, s, 1- and 3-OH), 5.07 (1H, br s, 1′-OH), and 5.18 (1H, br s, 3′-OH) confirmed the structure of **5** as a turriane derivative, a kind of (14-*p*,0-*o*)-cyclophane.[11] The *Z* geometry of the double bond was supported by the allylic carbons at δ 26.5 and vinylic carbons at δ 129.8 as

Table 1. ^{1}H NMR Data of Alkylresorcinols **1**−**8** (δ, multiplicity, *J*, Hz in parentheses)[a]

	1	2	3	4	5	6	7	8
H-2			6.25 s	6.23 s				
H-4	6.46 s	6.46 s	6.26 s	6.27 s	6.47 s	6.42 s	6.41 s	6.43 s
H-6	6.46 s	6.46 s	6.33 s	6.31s	6.47 s	6.42 s	6.41 s	6.43 s
H-2′	6.48 t (2.1)	6.48 t (2.1)	6.17 s	6.16 s	6.45 s	6.41 d (2.1)	6.21 s	6.43 s
H-4′	6.26 t (2.1)	6.26 t (2.1)	6.24 s	6.23 s	6.48 s	6.48 d (2.1)	6.32 s	6.48 s
H-6′	5.83 t (2.1)	5.82 t (2.1)	6.24 s	6.23 s				
H-1″	4.46 t (6.6)	4.46 t (6.4)	2.49 m	2.47 m	2.59 t (6.3)	2.57 t (6.6)	2.50 t (6.6)	2.58 t (6.4)
H-2″	1.72 m	1.72 m	1.56 m	1.53 m	1.58 m	1.59 m	1.60 m	1.60 m
H-3″−12″	1.27 m		1.25 brs				1.21 m	1.20 m
H-3″−6″, 11″, 12″		1.27 br s		1.26 br s	1.25 br s	1.23 m		
H-7″, 10″		2.00 m		2.02 m	1.95 m	1.94 m		
H-8″, 9″		5.33 m		5.35 m	5.32 m	5.30 m		
H-13″	1.45 m	1.45 m	1.56 m	1.53 m	1.36 m	1.38 m	1.30 m	1.27 m
H-14″	2.34 t (7.8)	2.35 t (7.8)	2.49 m	2.47 m	2.26 t (7.8)	2.29 t (7.8)	2.18 t (7.8)	2.29 t (7.8)
1-OH					4.73 s	4.59 s	5.28 br s	4.59 br s
3-OH			4.95 s	5.97 br s	4.73 s	4.59 s	5.28 br s	4.59 br s
1′-OH			4.95 s	5.97 br s	5.07 brs		5.78 br s	
3′-OH			4.95 s	5.97 br s	5.18 br s	5.16 br s	6.38 brs	5.25 br s
1-OCH_3			3.76 s	3.73 s				
1′-OCH_3						3.72 s		3.71 s

[a] **1** and **2** were measured in CD_3OD; **3**−**8** were measured in $CDCl_3$.

in **2** and **4**. According to the structure of tetramethoxyturriene and the possible biogenetic route to cyclophane,[11] the alkenyl chain pattern would be the same as that in **4** and **10**. Hence, (*Z*)-1,1′,3,3′-tetrahydroxyturri-8″-ene was deduced for the structure of dehydrograviphane (**5**).

Methyldehydrograviphane (**6**) was determined to have the molecular formula $C_{27}H_{36}O_4$ by HREIMS. The ^{1}H NMR data of **6** were almost the same as that of **5**. The difference was that **6** contained three hydroxyls at δ 4.59 (s, 1- and 3-OH) and 5.16 (br s, 3′-OH) and a methoxyl at δ 3.72 (3H, s, 1′-OCH_3) on the phenyl rings. The location of the methoxyl group was determined from its NOE cross-peak with H-2′ (δ 6.41) but not with H-4′ (δ 6.48). Thus, the structure of **6** was assigned as (*Z*)-1′-methoxy-1,3,3′-trihydroxyturri-8″-ene, and it was named methyldehydrograviphane. Compound **6** has been synthesized by Furstner et al.[15]

The molecular formulas of graviphane (**7**) and methylgraviphane (**8**) were established as $C_{26}H_{36}O_4$ and $C_{27}H_{38}O_4$ from the molecular ion peaks at *m*/*z* 412.2612 and 426.2773, respectively. By comparison of their spectroscopic data with those of **5** and **6**, they were found to be the saturated analogues of **5** and **6** with the same turriane ring system. Consequently, 1,1′,3,3′-tetrahydroxyturriane and 1′-methoxy-1,3,3′-trihydroxyturriane were assigned for the structures of graviphane (**7**) and methylgraviphane (**8**), respectively. Although the structures of **7** and **8** were proposed from a mixture obtained from an extract of *G. robusta* and synthesized by Ridley et al.,[11] this represents their first isolation as pure compounds from a natural source.

The alkylresorcinols **1**−**14** were all subjected to cytotoxic evaluation against MCF-7, NCI-H460, and SF-268 cell lines. They all showed marginal cytotoxicity (Table 3). The similar IC_{50} values of these compounds led us to conclude that there was no impact of alkyl chain, either cyclic or straight chain, on the cytotoxic activity of these alkylresorcinols.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance 300 and AMX 400 FT-NMR spectrometers; all chemical shifts are given in ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer using a direct inlet system.

Plant Material. The leaves of *Grevillea robusta* were collected on the campus of National Cheng Kung University, Tainan, Taiwan, in September 2003. The collection was identified by Professor C. S. Kuoh, Department of Life Sciences, National Cheng Kung University. A voucher specimen (No: PLW-0303) was deposited in the Herbarium of the same university.

Extraction and Isolation. The dried leaves of *G. robusta* (7.7 kg) were extracted with MeOH under reflux. The extract was concentrated under reduced pressure to give a dark green syrup. The syrup was suspended in H_2O and then partitioned with hexane. The concentrated hexane layer (150 g) was chromatographed on a silica gel column by eluting with a gradient of hexane−$(CH_3)_2CO$ (4:1 to pure $(CH_3)_2CO$) to yield eight fractions. Cytotoxic assay indicated that fractions 4−7 were active. Therefore, further chromatography of fraction 4 on a silica gel column eluting with a gradient of $CHCl_3$−MeOH (50:1 to pure MeOH) gave three active subfractions, A (105 mg), B (2.15 g), and C (825 mg). Repeated chromatography of A−C afforded small amounts of **6** and **8**, **13** and **14**, and **9** and **10**, respectively. Fraction 5 was chromatographed on silica gel eluting with a gradient of $CHCl_3$−MeOH (30:1 to pure MeOH) to give two active subfractions, D (35 mg) and E (180 mg). Repeated chromatography of D and E yielded **3** and **4** and **5** and **7**, respectively. Fraction 6 was chromatographed on silica gel eluting with a gradient of $CHCl_3$−MeOH (20:1 to pure MeOH) and gave active subfraction F (545 mg), which was subjected to chromatographic separation to give pure compounds **1** and **2**. Using the same separation procedure, fraction 7 was separated to produce one active subfraction, G (52 mg). Chromatographic purification of G gave **11** and **12**.

Gravicycle (1): white, amorphous powder; $[\alpha]_D$ +13.1 (c 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (4.1), 274 (3.5) nm; IR (KBr) ν_{max} 3346, 1597, 1502, 1459 cm^{-1}; ^{1}H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 428 (100, M^+), 410 (18), 369 (8), 260 (26), 232 (12), 124 (18); HREIMS *m*/*z* 428.2565 $[M]^+$ (calcd for $C_{26}H_{36}O_5$ 428.2563).

Acylation of 1 with MTPACl to 1a. (*R*)- or (*S*)-MTPACl (10 mg) was added to a pyridine solution (0.2 mL) containing **1** (1.0 mg). The reaction mixture was stirred at room temperature for 4 h, then evaporated to dryness. The residue was washed with water, extracted with EtOAc, and dried with anhydrous $MgSO_4$. The filtered organic solution was concentrated and subjected to chromatography on silica gel using hexane−EtOAc (10:1) as eluent. Pure **1a**-(*S*)-MTPA (2.7 mg) or **1a**-(*R*)-MTPA (2.5 mg) was thus obtained. ^{1}H NMR data for **1a**-(*S*)-MTPA: δ 1.26 (20H, m), 1.35 (2H, m), 1.95 (2H, m, H-2″), 2.38 (2H, t, J = 7.8 Hz), 3.31 (3H, s), 3.42 (3H, s), 3.53 (3H, s), 3.67 (3H, s), 5.94 (1H, t, J = 6.6 Hz, H-1″), 6.08 (1H, s), 6.52 (1H, s), 6.60 (1H, s), 7.00 (1H, s, H-4), 7.09 (1H, s, H-6), 7.2−7.7 (20H, m); for **1a**-(*R*)-MTPA: δ 1.25 (20H, m), 1.38 (2H, m), 1.88 (2H, m, H-2″), 2.39 (2H, t, J = 8.1 Hz), 3.36 (6H, S), 3.50 (3H, s), 3.68 (3H, s), 6.02 (1H, t, J = 6.6 Hz, H-1″), 6.12 (1H, s), 6.56 (1H, s), 6.60 (1H, s), 7.10 (2H, s, H-4 and -6), 7.2−7.7 (20H, m).

Dehydrogravicycle (2): white, amorphous powder; $[\alpha]_D$ +13.3 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.1), 275 (3.3) nm; IR (KBr) ν_{max} 3396, 1598, 1505, 1458 cm^{-1}; ^{1}H NMR data, Table 1; ^{13}C

Table 2. ^{13}C NMR Data of Alkylresorcinols **1**−**8**[a]

	1	2	3	4	5	6	7	8
C-1	151.8	151.8	160.7	160.6	154.5	153.5	154.3	153.5
C-2	130.4	130.4	98.7	98.8	103.4	107.8	104.4	107.3
C-3	151.8	151.8	156.4	156.4	154.5	153.5	154.3	153.5
C-4	106.7	106.7	107.9	108.1	108.3	107.6	108.2	107.6
C-5	143.9	143.9	145.8	145.8	146.8	144.7	146.4	144.8
C-6	106.7	106.7	106.8	106.8	108.3	107.6	108.2	107.6
C-1′	160.8	160.8	156.6	156.5	156.4	159.9	155.7	160.0
C-2′	102.7	102.7	100.1	100.3	100.9	97.5	100.9	97.5
C-3′	159.3	159.3	156.6	156.5	158.2	157.9	157.7	158.0
C-4′	109.9	109.9	108.0	108.1	109.8	109.3	109.8	109.0
C-5′	146.0	146.0	146.2	145.8	147.5	148.1	147.5	148.1
C-6′	105.3	105.2	108.0	108.1	105.7	108.7	106.6	108.5
C-1″	75.3	75.4	35.8	36.0	36.0	35.1	35.5	35.6
C-2″	40.0	40.0	31.1	31.1	30.8	29.9	30.3	30.4
C-3″	26.4	26.4	29.2−29.6	29.1−29.8	27.3−29.9	27.0−29.4	26.8−29.4	26.8−29.7
C-4″−6″	27.9−30.2	27.9−30.2	29.2−29.6	29.1−29.8	27.3−29.9	27.0−29.4	26.8−29.4	26.8−29.7
C-7″, 10″	27.9−30.2	27.6	29.2−29.6	27.1	26.5	26.7	26.8−29.4	26.8−29.7
C-8″, 9″	27.9−30.2	129.8	29.2−29.6	129.8	129.8	129.9	26.8−29.4	26.8−29.7
C-11″, 12″	27.9−30.2	27.9−30.2	29.2−29.6	29.1−29.8	27.3−29.9	27.0−29.4	26.8−29.4	26.8−29.7
C-13″	32.2	32.2	31.1	31.1	31.3	31.4	31.2	31.4
C-14″	37.0	37.0	36.0	36.0	34.2	33.4	33.6	33.6
1-OCH_3			55.2	55.3				
1′-OCH_3						55.9		55.9

[a] **1** and **2** were measured in CD_3OD; **3**−**8** were measured in $CDCl_3$.

Table 3. Cytotoxicity of **1**−**14** toward Three Cancer Lines[a]

	IC_{50} (μM)		
compound	MCF-7	NCI-H460	SF-268
1	30.6 ± 0.3	27.0 ± 0.6	32.1 ± 0.8
2	30.0 ± 0.1	27.9 ± 1.8	31.3 ± 0.4
3	29.4 ± 1.2	28.7 ± 1.4	29.4 ± 1.0
4	29.8 ± 0.3	27.0 ± 2.1	30.2 ± 1.3
5	30.7 ± 1.5	25.4 ± 2.9	33.4 ± 1.1
6	29.3 ± 0.3	27.9 ± 3.9	29.1 ± 1.1
7	28.6 ± 3.2	22.8 ± 1.3	27.7 ± 1.5
8	30.8 ± 1.9	28.9 ± 1.5	31.1 ± 0.1
9	32.4 ± 0.1	24.0 ± 5.0	32.7 ± 1.2
10	31.8 ± 0.8	28.0 ± 3.2	33.1 ± 0.4
11	29.2 ± 0.8	28.8 ± 0.3	29.8 ± 0.2
12	28.6 ± 1.5	27.7 ± 1.2	30.6 ± 0.8
13	37.0 ± 1.1	34.2 ± 1.2	39.8 ± 0.3
14	37.1 ± 1.9	35.4 ± 1.7	39.2 ± 0.7

[a] Values are mean ± SD (n = 3−8). MCF-7 = human breast tumor cell line; NCI-H460 = human lung tumor cell line; SF-268 = human central nervous system tumor cell line.

NMR data, Table 2; EIMS *m*/*z* (rel int) 426 (100, M^+), 369 (12), 260 (40), 246 (14), 229 (15), 163 (10), 149 (8), 137 (12), 123 (34); HREIMS *m*/*z* 426.2403 $[M]^+$ (calcd for $C_{26}H_{34}O_5$ 426.2406).

Bisgravillol (3): white, amorphous powder; UV ($CHCl_3$) λ_{max} (log ϵ) 242 (3.6), 280 (3.5) nm; IR (KBr) ν_{max} 3362, 1597, 1464 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 428 (49, M^+), 180 (4), 163 (4), 151 (15), 138 (100), 124 (42); HREIMS *m*/*z* 428.2924 $[M]^+$ (calcd for $C_{27}H_{40}O_4$ 428.2927).

Dehydrobisgravillol (4): colorless syrup; UV ($CHCl_3$) λ_{max} (log ϵ) 242 (3.6), 281 (3.5) nm; IR (KBr) ν_{max} 3372, 1598, 1463 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 426 (52, M^+), 246 (6), 217 (19), 205 (8), 189 (14), 177 (24), 163 (32), 138 (100), 124 (56); HREIMS *m*/*z* 426.2773 $[M]^+$ (calcd for $C_{27}H_{38}O_4$ 426.2770).

Diethyldisulfide Adduct 4a from 4. Acetyl chloride (12 mg) was added to a pyridine solution (0.5 mL) containing dehydrobisgravillol (**4**) (3.4 mg). The solution was stirred at room temperature for 4 h. The reaction mixture was quenched with water and extracted with EtOAc. The organic extract was dried with $MgSO_4$ and chromatographed on silica gel eluting with hexane−EtOAc (3:1) to give pure acetylated dehydrobisgravillol. The acetylated dehydrobisgravillol was added to a hexane (0.5 mL) solution that contained a catalytic amount of iodine. Diethyldisulfide (0.1 mL) was then added, and the solution was heated at 50 °C for 12 h. After evaporating the solvent, the residue was chromatographed on a silica gel column eluting with hexane−EtOAc (2:1) to obtain the diethyldifulfide adduct **4a**: EIMS *m*/*z* (rel int) 674 (1, M^+), 337 (100), 320 (38).

Dehydrograviphane (5): colorless syrup; UV ($CHCl_3$) λ_{max} (log ϵ) 243 (3.8), 281 (3.6), 324 (2.8) nm; IR (KBr) ν_{max} 3408, 1621, 1569, 1455 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 410 (100, M^+), 246 (17), 229 (15), 161 (5), 149 (3), 123 (8); HREIMS *m*/*z* 410.2455 $[M]^+$ (calcd for $C_{26}H_{34}O_4$ 410.2457).

Methyldehydrograviphane (6): white, amorphous powder; UV (MeOH) λ_{max} (log ϵ) 244 (4.0), 280 (3.9), 324 (2.9) nm; IR (KBr) ν_{max} 3405, 1604, 1585, 1458 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 424 (100, M^+), 260 (14), 243 (11), 166 (6), 149 (9), 137 (6); HREIMS *m*/*z* 424.2610 $[M]^+$ (calcd for $C_{27}H_{36}O_4$ 424.2613).

Graviphane (7): colorless syrup; UV ($CHCl_3$) λ_{max} (log ϵ) 244 (3.8), 281 (3.6) nm; IR (KBr) ν_{max} 3408, 1621, 1573, 1455 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 412 (100, M^+), 246 (16), 229 (14), 211 (11), 197 (5), 149 (13), 124 (9), 123 (6); HREIMS *m*/*z* 412.2612 $[M]^+$ (calcd for $C_{26}H_{36}O_4$ 412.2614).

Methylgraviphane (8): white, amorphous powder; UV (MeOH) λ_{max} (log ϵ) 248 (3.9), 280 (3.8), 323 (2.5) nm; IR (KBr) ν_{max} 3413, 1584, 1459 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* (rel int) 426 (100, M^+), 260 (10), 243 (9), 137 (5); HREIMS *m*/*z* 426.2773 $[M]^+$ (calcd for $C_{27}H_{38}O_4$ 426.2770).

Diethyldisulfide Adduct 10a from 10. A mixture of **9** and **10** (15 mg), (trimethylsilyl)diazomethane (2.0 M solution in hexane, 0.2 mL), and MeOH (0.8 mL) in benzene (3 mL) was placed in a sealed tube and heated at 65 °C for 6 h. After the excess (trimethylsilyl)-diazomethane was decomposed with acetic acid, the reaction mixture was evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with hexane−EtOAc (10:1) to give pure methylated dehydrorobustol A (7.5 mg). The methylated dehydrorobustol A was added to dry liquid NH_3 (5 mL). Sodium (500 mg) was then added in small pieces, and the solution was continuously stirred until it retained an intense blue color for 2 h. The coolant was withdrawn, and the mixture was stirred for another 2 h in a Dewar flask without additional cooling. A surplus of NH_4Cl was added, and the NH_3 was removed in a stream of N_2. The residue was purified by column chromatography on silica gel eluting with hexane−EtOAc (1:1) to give **10a** (5.1 mg): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.27 (12H, m, H-3″−6″, -11″, and -12″), 1.58 (4H, m, H-2″ and -13″), 2.00 (4H, m, H-7″ and -10″), 2.52 (4H, m, H-1″ and -14″), 3.76 (3H, s, 3-OCH_3), 3.78 (6H, s, 1′- and 3′-OCH_3), 4.74 (1H, s, 1-OH), 5.32 (2H, m, H-8″ and -9″), 6.23 (1H, s, H-2), 6.25 (1H, s, H-6), 6.30 (1H, s, H-2′), 6.32 (1H, s, H-4), 6.35 (2H, s, H-4′ and -6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.1 (C-7″ and -10″), 29.1−29.7 (C-3″−6″, -11″, and -12″), 31.1 and 31.3 (C-2″ and -13″), 36.0 and 36.3 (C-1″ and -14″), 55.2 (1′, 3, and 3′-OCH_3), 97.5 (C-2′), 98.6 (C-2), 106.5 (C-4′ and -6′), 106.8 (C-4), 107.8 (C-6), 129.9 (C-8″ and -9″), 145.4 (C-5), 145.8 (C-5′),

156.4 (C-1), 160.6 (C-1′ and -3′), 160.8 (C-3); EIMS *m*/*z* (rel int) 454 (25, M^+), 152 (100), 138 (43). Using the same procedure for the preparation of **8a** from **8**, acylation with acetyl chloride and addition with diethyldisulfide of **10a** (5.1 mg) gave **10b** (4.3 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (6H, t, J = 7.3 Hz, 2 × SCH_2CH_3), 1.32 (16H, br s, H-3″−7″ and 10″−12″), 1.60 (4H, m, H-2″ and -13″), 2.28 (3H, s, 1-$OCOCH_3$), 2.52 (4H, q, J = 7.3 Hz, 2 × SCH_2CH_3), 2.56 (4H, m, H-1″ and -14″), 2.74 (2H, m, H-8″ and -9″), 3.78 (9H, s, 1′-, 3-, and 3′-OCH_3), 6.30 (1H, s, H-2′), 6.34 (2H, s, H-4′ and -6′), 6.46 (1H, s, H-2), 6.51 (1H, s, H-6), 6.60 (1H, s, H-4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.1 (2 × SCH_2CH_3), 21.2 (1-$OCOCH_3$), 26.3 (2 × SCH_2CH_3), 29.1−29.7 (C-3″−7″ and 10″−12″), 31.4 (C-2″ and -13″), 35.9 (C-1″), 36.3 (C-14″), 51.1 (C-8″ and -9″), 55.2 (1′- and 3′-OCH_3), 55.3 (3-OCH_3), 97.5 (C-2′), 104.6 (C-2), 106.5 (C-4′ and -6′), 111.9 (C-4), 113.8 (C-6), 145.4 (C-5 and -5′), 151.4 (C-1), 160.2 (C-3), 160.7 (C-1′ and -3′), 169.5 (1-$OCOCH_3$); EIMS *m*/*z* (rel int) 618 (10, M^+), 369 (21), 309 (100), 295 (41), 152 (46), 151 (37), 137 (28).

Cytotoxicity Assay. The cytotoxicity assay was carried out according to the procedure described in the literature.[16]

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