Cytotoxic Grifolin Derivatives Isolated from the Wild Mushroom *Boletus pseudocalopus* (Basidiomycetes)

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Activity-guided purification of a MeOH extract of the Korean wild mushroom *Boletus pseudocalopus* afforded three new grifolin derivatives, **1**–**3**, along with four known phenolic compounds **4**–**7**. Their structures were established by a combination of ^{1}H- and ^{13}C-NMR, NOESY, and extensive two-dimensional NMR spectroscopic experiments such as gCOSY, gHSQC, gHMBC, and ROESY. The major metabolites **4** and **5** were subjected to reduction to provide the side chain-reduced compounds **8** and **9** for biological testing. All of the compounds except compound **6** showed anticancer activities in the range of IC_{50} 3.5–11.0 μg/ml against human lung carcinoma A549 and mouse melanoma B16F1 cell lines. In addition, all compounds showed moderate radical-scavenging activities determined by DPPH assay.

Introduction. – Mushrooms (macrofungi) accumulate a variety of cytotoxic secondary metabolites including polyphenols, terpenoids, and alkaloids [1–5]. Medicinal mushrooms such as *Ganoderma lucidum*, *Phellinus linteus,* and *Coriolus versicolor*, have an established history of use in traditional Asian therapies [6][7]. Several polysaccharides and polysaccharide conjugates have been commercialized for the clinical treatment of patients undergoing anticancer therapy. Irofulven, derived from antitumor sesquiterpene illudins, possesses strong cytotoxic effects against a wide variety of solid tumors [2][8].

In the search for biologically active compounds from natural sources [9][10], we have collected hundreds of wild mushroom species in the mountainous areas during the hot humid summer and prepared MeOH extracts from them for anticancer-activity tests. Among them, the extracts from *Boletus pseudocalopus* showed strong cytotoxic activities against human lung cancer, mouse melanoma, and human melanoma cell lines with IC_{50} values of 20, 15, and 40 μg/ml, respectively, as evaluated by the sulforhodamine B (SRB) colorimetric method. This mushroom is recognized by its red fruiting bodies and the blue staining, when the fruit-bodies are cut or brushed, presumably due to the characteristic bolete pigments like xerocomic acid, variegatic acid, and variegatorubin [11][12]. Phytochemicals in this species have been rarely investigated, while the Boletales like *B. satanas*, *B. erythropus*, and *B. curtisii* have been reported to produce cytotoxic glycoprotein bolesatine, which shows inhibitory effect of protein synthesis in radiation-induced thymic lymphosarcoma cell line (IC_{50} 0.6 μg/ml) [13], a water-soluble β-glucan [14], and yellow pigments curtisins [15], respectively.

Here, we report the isolation and structure determination of new cytotoxic metabolites **1**–**3** together with known grifolin derivatives **4**–**7** from the wild Korean mushroom *B. pseudocalopus.* In addition, their cytotoxic effects against cancer cell lines

(human lung, human melanoma, and mouse melanoma) and radical-scavenging activities are reported.

Results and Discussion. – 1. *Isolation and Structure Elucidation*. Fresh wild *B. pseudocalopus* mushrooms (1.1 kg) were extracted twice with MeOH. The extracts were fractionated by a silica-gel flash column chromatography, and their cytotoxicities against human lung cancer A549, mouse melanoma B16F1, and human melanoma SK-Mel-2 cell lines evaluated by the SRB colorimetric cell-growth inhibition measurements. The active fractions were further purified by C_{18} MPLC, followed by preparative C_{18} HPLC to afford 5.0, 3.1, 4.6, 2.9, and 5.8 mg of compounds **1**, **2**, **3**, **6**, and **7** as minor metabolites, respectively, and 2.0 and 1.3 g of compounds **4** and **5** as major metabolites, respectively (*Fig. 1*).

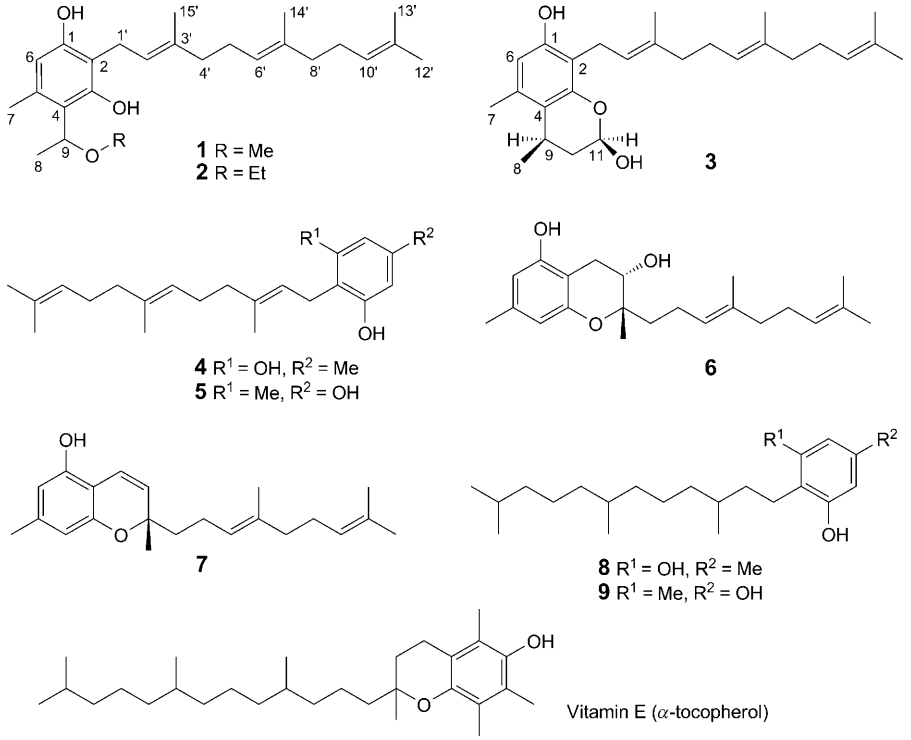

Fig. 1. *Chemical structures of grifolin derivatives* **1**–**9** *isolated from* Boletus pseudocalopus*, and vitamin E* (*α-tocopherol*)

Compound **1** was obtained as a pale yellow oil with a specific optical rotation of +2.8 ($c = 0.36$, MeOH). The molecular formula was determined to be $C_{25}H_{38}O_3$ as the high-resolution time-of-flight mass spectrum (HR-TOF-MS) showed its deprotonated molecular ion $[M-H]^-$ at m/z 385.2740. The IR spectral absorptions at $\tilde{\nu}_{max}$ 3345, 1622, and 1600 cm^{-1}, and the UV absorption (MeOH) at λ_{max} 231 and 273 nm indicated the presence of a phenolic group. The ^{1}H-NMR spectrum in $CDCl_3$ gave signals for five Me groups as *singlet*s at δ 1.58, 1.59, 1.67, 1.81, and 2.15, for one Me group as *doublet* at δ

1.47 ($J=6.8$), and signals of a MeO group at δ 3.36, of CH H-atom at δ 4.69 (q, $J=6.8$), of three olefinic H-atoms at δ 5.06–5.08 (m, 2 H) and 5.29 (tq, $J=6.0, 0.8, 1$ H), and of an aromatic H-atom as *singlet* at δ 6.20. The ^{13}C-NMR spectrum showed the presence of two O-bearing aromatic C-atoms (δ 154.3 and 155.1). The characteristic appearance of a farnesyl group was observed in the ^{1}H- and ^{13}C-NMR spectra (*Table 1*). In the HMBC spectrum, important long-range C,H correlations were observed, which served to locate the side chains and the MeO group: H–C(9) to C(3), C(4), C(5), and C(8); H–C(1′) to C(2), C(3), C(2′), and C(3′); Me(7) to C(4), C(5), and C(6); and the MeO H-atoms to C(9) (*Fig. 2*). The interpretation of the 2D-NMR spectra (^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY) allowed all ^{1}H- and ^{13}C-NMR assignments; thus, the structure of compound **1** was determined to be 4-(1-methoxyethyl)-5-methyl-2-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol.

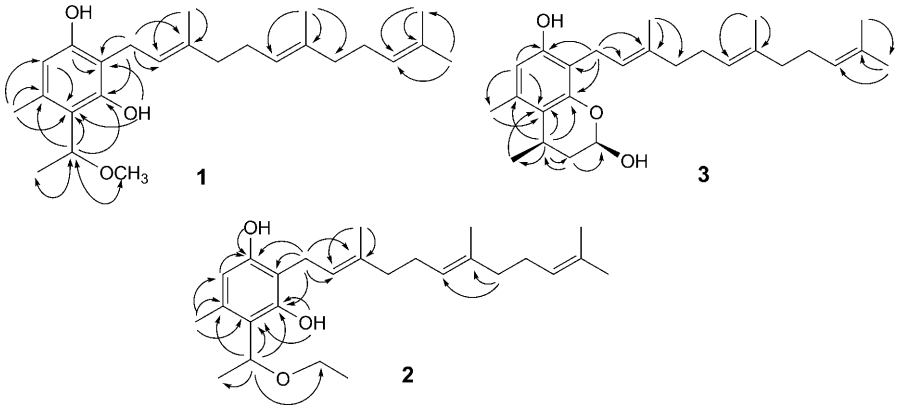

Fig. 2. *HMBC Correlations* (H→C) *in* **1**–**3**

Compound **2** was obtained as a pale yellow oil ($[\alpha]_D^{20}=+0.97$ ($c=0.31$, MeOH)). The molecular formula was determined to be $C_{26}H_{40}O_3$ by negative-ion HR-TOF-MS (m/z 399.2869 ($[M-H]^-$). The IR and UV spectra of compound **2** were observed to be almost identical with those of compound **1**. The ^{1}H- and ^{13}C-NMR spectra of compound **2** were similar with those of compound **1**, except the signals of the EtO group observed at δ(H) 1.26 (δ(C) 15.6) and δ(H) 3.53–3.61 (δ(C) 65.1) of compound **2** instead those of the MeO group of compound **1**. Thus, the structure of compound **2** was deduced to be 4-(1-ethoxyethyl)-5-methyl-2-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol. All ^{1}H and ^{13}C signals were assigned from the interpretation of extensive 2D NMR (HSQC, HMBC, and ROESY) experiments (*Table 1*).

Compound **3** was obtained as a pale yellow oil with a molecular formula of $C_{26}H_{38}O_3$ determined by the HR-TOF-MS spectrum. It showed a specific optical rotation of $[\alpha]_D^{20}=-3.8$ ($c=0.42$, MeOH). The IR ($\tilde{\nu}_{max}$ 3330, 1604, and 1450 cm^{-1}) and UV (λ_{max} 235 and 283 nm) spectra of compound **3** indicated the presence of a OH group and an aromatic ring. The ^{1}H-NMR spectrum of **3** in $CDCl_3$ showed the characteristic signals for a farnesyl group and one aromatic H-atom signal. In the ^{1}H,^{1}H-COSY spectrum, the CH H-atom (δ 3.05 (br. *quint.*, $J=7.2$)) was observed to be coupled with the Me H-atoms (δ 1.24 (d, $J=7.2$)) and the CH_2 H-atoms (δ 1.78–1.85 (m) and 2.02–2.05 (m)),

Table 1. *^{1}H- and ^{13}C-NMR Data of the Compounds* **1**–**3** *in $CDCl_3$.* δ in ppm; J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		155.1		154.8		153.9
2		112.6		112.4		112.3
3		154.3		154.2		150.8
4		116.4		116.9		117.6
5		134.1		133.7		134.7
6	6.20 (*s*)	109.6	6.20 (*s*)	109.4	6.30 (*s*)	110.8
7	2.15 (*s*)	19.4	2.16 (*s*)	19.5	2.22 (*s*)	18.9
8	1.47 (*d*, J=6.8)	20.8	1.48 (*d*, J=6.8)	21.1	1.24 (*d*, J=7.2)	21.8
9	4.69 (*q*, J=6.8)	78.2	4.80 (*q*, J=6.8)	76.0	3.05 (br. *q*, J=7.2)	27.0
10					2.02–2.05 (*m*), 1.78–1.85 (*m*)	36.7
11					5.53 (br. *d*, J=8.8)	92.0
1′	3.41 (*d*, J=7.2)	22.2	3.42 (*d*, J=7.0)	22.4	3.40 (*d*, J=6.8)	22.6
2′	5.29 (*tq*, J=6.0, 0.8)	122.4	5.31 (*tq*, J=7.0, 1.0)	122.3	5.25 (br. *t*, J=7.0)	122.4
3′		138.5		138.3		138.2
4′	2.04–2.07 (*m*)	40.0	2.04–2.08 (*m*)	40.1	2.04–2.08 (*m*)	40.5
5′	2.08–2.11 (*m*)	26.7	2.10–2.13 (*m*)	26.8	2.07–2.12 (*m*)	26.6
6′	5.06–5.08 (*m*)	124.0	5.08–5.10 (*m*)	123.9	5.07 (br. *t*, J=6.8)	124.0
7′		135.7		135.5		135.7
8′	1.94–1.98 (*m*)	39.9	1.96–2.00 (*m*)	40.0	1.94–1.98 (*m*)	39.9
9′	2.02–2.05 (*m*)	26.9	2.03–2.06 (*m*)	27.0	2.02–2.05 (*m*)	26.9
10′	5.06–5.08 (*m*)	124.6	5.08–5.10 (*m*)	124.5	5.09 (br. *t*, J=6.8)	124.6
11′		131.5		131.3		131.6
12′	1.67 (br. *s*)	25.9	1.69 (*d*, J=1.2)	26.0	1.67 (*s*)	25.9
13′	1.59 (*s*)	17.9	1.61 (*s*)	18.0	1.59 (*s*)	17.9
14′	1.58 (br. *s*)	16.2	1.60 (*d*, J=0.8)	16.4	1.57 (*s*)	16.3
15′	1.81 (br. *s*)	16.4	1.82 (*d*, J=1.0)	16.6	1.80 (*s*)	16.5
MeO–C(9)	3.36 (*s*)	57.1				
EtO–C(9)			3.53–3.61 (*m*), 1.26 (*t*, J=7.0)	65.1 15.6		

which were in turn coupled with the CH H-atom (δ 5.53 (br. *d*, J=8.8)). Based on the coupling constants, the two CH H-atoms were assigned to be axial. These H-atoms were correlated with the corresponding C-atoms in the HSQC spectrum as follows: δ(H) 3.05 to δ(C) 27.0; δ(H) 1.24 to δ(C) 21.8; δ(H) 1.78–1.85 and 2.02–2.05 to δ(C) 36.7; and δ(H) 5.53 to δ(C) 92.0. In the HMBC experiment, the H-atom with the signal at δ 3.05 (H–C(9)) was correlated with the C-atoms with signals at δ 21.8, 36.7, 117.6, 134.7, and 150.8; other important correlations were observed between CH_2(10) and C(8), C(9), and C(11); and H–C(1′) and C(1), C(2), C(3), C(2′), and C(3′) (*Fig. 2*). Thus, the structure of compound **3** was determined to be (2*R**,4*R**)-3,4-dihydro-4,5-dimethyl-8-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]-2*H*-[1]benzopyran-2,7-diol.

Compound **4**, the major metabolite of this mushroom, was obtained as a brown solid. Its molecular formula was deduced to be $C_{22}H_{31}O_2$ based on the positive-ion HR-TOF-MS spectrum (*m*/*z* 329.2482, $[M+H]^+$). Upon extensive spectroscopic analysis, it was determined to be grifolin, previously isolated from the inedible mushroom

Grifola confluens with antibiotic activities [16][17] and from *Albatrellus ovinus* with antioxidant activities [18]. The second major metabolite **5** was obtained as a brown solid and determined to be neogrifolin [18], an isomer of grifolin. Compound **6** was obtained as a brown oil and determined to be 2-(4,8-dimethylnona-3,7-dienyl)-3,4-dihydro-2,7-dimethyl-2*H*-[1]benzopyran-3,5-diol [19]. Compound **7** was obtained as a brown oil and determined to be 2-((2*E*,6*E*)-4,8-dimethylnona-3,7-dienyl)-2,7-dimethyl-2*H*-[1]benzopyran-5-ol, a dehydrated form of compound **6**, which was previously described in the literature as confluentin isolated from *Albatrellus* spp. [20].

To see the effect of the relatively rigid structural feature of the farnesyl groups on the biological activity, compounds **8** and **9** were prepared from the hydrogenation of compounds **4** and **5** with H_2 gas in the presence of Pd/C catalyst at room temperature in MeOH, respectively.

2. *Biological Studies.* The cytotoxic effects of the isolates **1**–**7**, and the reduced compounds **8** and **9** were evaluated by SRB colorimetric method against human lung carcinoma, mouse melanoma, and human melanoma cell lines. The IC_{50} values of the compounds, except compound **6**, were within a range of 5.0–11.0 and 3.5–7.3 μg/ml against human lung carcinoma A549 and mouse melanoma B16F1, respectively, comparable to cisplatin, which was used as a positive control (*Table 2*). The new isolates **1**–**3** showed relatively stronger cytotoxic effects against human melanoma SK-Mel-2 than the other compounds. It is interesting to note that vitamin E (α-tocopherol), a structurally related antioxidant, showed no cytotoxic effects against all the cancer cell lines tested below a concentration of 40 μg/ml. The radical-scavenging activities of compounds **1**–**9** were also evaluated by using the diphenyl-*p*-picrylhydrazyl (DPPH) assay method. All compounds showed slightly lower radical-scavenging activities compared to vitamin E (*Table 2*). Hydrogenated compounds **8** and **9** did not significantly increase or decrease cytotoxicity or radical-scavenging activities over the mother compounds, grifolin (**4**) and neogrifolin (**5**). Recently, **4** was reported to

Table 2. *Cytotoxic and Free-Radical-Scavenging Activities of Compounds* **1**–**9** (IC_{50} [μg/ml] ± SD)[a])

Compound	Cell line[b])			DPPH Radical[c])
	A549	B16F1	SK-Mel-2	
1	8.5 ± 0.6	7.3 ± 0.7	16.9 ± 1.8	72.4 ± 7.8
2	9.0 ± 0.9	4.3 ± 0.2	11.9 ± 1.4	63.1 ± 6.2
3	5.0 ± 0.7	3.5 ± 0.7	13.7 ± 1.2	67.4 ± 4.5
4	10.5 ± 1.3	6.1 ± 0.2	> 40	43.8 ± 6.8
5	10.5 ± 0.7	5.4 ± 0.4	> 40	11.1 ± 1.8
6	> 40	> 40	> 40	75.9 ± 3.3
7	11.0 ± 1.1	6.0 ± 0.8	8.0 ± 1.1	67.4 ± 5.2
8	7.5 ± 0.8	6.5 ± 1.1	> 40	28.6 ± 3.5
9	5.0 ± 0.3	4.1 ± 0.4	> 40	14.6 ± 2.6
α-Tocopherol	> 40	> 40	> 40	4.8 ± 0.8
Cisplatin	9.5 ± 0.5	5.2 ± 0.3	10.0 ± 0.9	–

[a]) Values are means of three independent experiments with standard deviations. [b]) A549: Human lung carcinoma; B16F1: mouse melanoma; SK-Mel-2: human skin cancer cell line. [c]) DPPH: Diphenyl-*p*-picrylhydrazyl; '–' indicates that the sample was not tested.

inhibit tumor cell growth by inducing apoptosis *in vitro* [21] and induce cell-cycle arrest in the G1 phase *via* the ERK1/2 pathway [1]. The derivatives of **4** with cytotoxic effects as well as radical-scavenging activities would serve as antitumor compounds.

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Experimental Part

General. TLC: silica-coated plastic plates (*Merck*, *Kieselgel 60 F_{254}*, 0.25 mm); spots were detected under a UV lamp (254 nm) or by heating after spraying with a soln. of anisaldehyde-H_2SO_4 in EtOH. Column chromatography (CC): silica gel *60* (*Merck*, 70–230 mesh). *C_{18}* Medium-pressure liquid chromatography (MPLC): *YAMAZEN F540* pump system using *C_{18} LiChroprep RP-18* (25–40 μm, 30 i.d. × 300 mm, *Merck*) at a flow rate of 10 ml/min. Prep. HPLC: *Waters 600* system with a photodiode array detector *996* using *C_{18} Pegasil* (*Senshu Pak*, 20 i.d. × 250 mm); flow rate 7 ml/min and detection at 210 nm. Optical rotation: *Perkin-Elmer 341* LC polarimeter. UV Spectra: *Shimadzu UV-2401 PC* spectrometer; λ_{max} in nm (log ε). IR Spectra: *Perkin-Elmer BX* FT-IR spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian Mercury 400* NMR spectrometer at 400 and 100 MHz, resp., in $CDCl_3$, referenced in residual solvent signals (δ(H) 7.26 and δ(C) 77.24 for $CDCl_3$; δ(H) 3.30 and δ(C) 49.0 for CD_3OD). HR-TOF-MS: *Waters LCT Premier* mass spectrometer on an electron spray ionization (ESI) mode coupled to a *Waters AQUITY* UPLC system and data acquisition using *MassLynx* software, version 4.0; in *m*/*z*. Optical density for 96-well microplates: *Tecan Sunrise* microplate reader.

Plant Material. The fruiting bodies of the mushroom *Boletus pseudocalopus* were collected in the Jirisan National Forest in the republic of Korea in July, 1998. The mushroom was identified by Dr. *Yang Seob Kim* at the National Institute Agricultural Science and Technology in Suwon, Republic of Korea. A voucher specimen (No. SM 5345) was deposited with the natural-product chemistry laboratory of Kongju National University, Republic of Korea.

Extraction and Isolation. The fresh fruiting bodies of the fresh mushroom (1.0 kg) were soaked in MeOH (1 l) and left for two weeks at r.t. The extract was decanted, and this was repeated one more time. The combined extracts were concentrated under vacuum to yield a brown oily syrup (46.5 g). The crude extract was chromatographed on a silica-gel column (50 i.d. × 350 mm) with elution by a mixture of hexane and AcOEt of increasing polarity to yield 17 fractions. *Fr. 3* (1.1 g; hexane/AcOEt 8:2) was further purified by *C_{18}* MPLC (MeOH/H_2O 6:4 to 10:0 for 200 min) to yield ten subfractions. *Fr. 3.5* (26 mg) was purified by *C_{18}* HPLC (MeOH/H_2O 9:1 to 10:0 for 80 min) to yield **1** (5.0 mg), eluting at 22.5 min. *Fr. 3.7* (50 mg) was purified by *C_{18}* HPLC (MeOH/H_2O 9:1 to 10:0 for 80 min) to yield **2** (3.1 mg), eluting at 37.7 min. *Fr. 4* (3.2 g, hexane/AcOEt 8:2) was further purified using *C_{18}* MPLC (MeOH/H_2O 6:4 to 10:0 for 200 min) to yield four subfractions. The concentration of the *Fr. 4.2* yielded **4** (= *grifolin*; 2.0 g), and the *Fr. 4.4* (271 mg) was purified by *C_{18}* HPLC (MeOH/H_2O 9:1 to 10:0 for 140 min) to provide **6** (2.9 mg), eluting at 22.4 min, and compound **7** (5.8 mg), eluting at 40.0 min. *Fr. 5* (800 mg, hexane/AcOEt 7:3) was further purified using *C_{18}* MPLC (MeOH/H_2O 6:4 to 10:0 for 200 min) to yield eight subfractions. The *Fr. 5.4* (33 mg) was purified by *C_{18}* HPLC (MeOH/H_2O 9:1 to 10:0 for 80 min) to yield **3** (4.6 mg) eluting at 12.3 min. *Fr. 6* (3.6 g, hexane/AcOEt 6:4) was subjected to *C_{18}* MPLC (MeOH/H_2O 6:4 to 10:0) to yield four subfractions. Concentration of *Fr. 6.3* yielded **5** (= neogrifolin; 1.7 g).

Hydrogenation of **4**. Compound **4** (54.6 mg, 0.166 mmol) was stirred at r.t. overnight under a H_2 atmosphere in the presence of Pd/C (5 mg) in MeOH (5 ml). Filtration through a short column of silica gel and concentration provided **8** (54.3 mg).

Hydrogenation of **5**. The reaction was carried out in the same way as described for compound **4** except for the use of **5** (42.4 mg, 0.129 mmol) to provide **9** (42.1 mg).

*4-(1-Methoxyethyl)-5-methyl-2-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol* (**1**). Pale yellow oil. $[\alpha]_D^{20} = +2.8$ ($c = 0.36$, MeOH). UV (MeOH): 204 (4.37), 231 (sh., 3.7), 273 (2.79). IR

(neat, KBr plate): 3345, 2945, 1622, 1600, 1449, 1269, 1027, 739. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-TOF-MS: 385.2740 ($[M-H]^-$, $[C_{25}H_{38}O_3-H]^-$; calc. 385.2743).

*4-(1-Ethoxyethyl)-5-methyl-2-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol* (**2**). Pale yellow oil. $[\alpha]_D^{20} = +0.97$ ($c = 0.31$, MeOH). UV (MeOH): 207 (4.66), 234 (sh., 3.9), 274 (2.91). IR (neat, KBr plate):3317, 2974, 1622, 1600, 1450, 1226, 1164, 1028, 832. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-TOF-MS: 399.2869 ($[M-H]^-$, $[C_{26}H_{40}O_3-H]^-$; calc. 399.2899).

*3,4-Dihydro-4,5-diemethyl-8-[(2*E*,6*E*)-3,7,11-trimethyldodec-2,6,10-trienyl]-2*H*-[1]benzopyran-2,7-diol* (**3**). Pale yellow oil. $[\alpha]_D^{20} = -3.8$ ($c = 0.42$, MeOH). UV (MeOH): 208 (4.78), 235 (sh., 3.01), 283 (3.15). IR (neat, KBr plate): 3330, 2943, 1604, 1450, 1146, 1096, 1027, 737. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-TOF-MS: 397.2725 ($[M-H]^-$, $[C_{26}H_{38}O_3-H]^-$; calc. 397.2743).

5-Methyl-2-(3,7,11-trimethyldodec)benzene-1,3-diol (**8**). Pale yellow solid. M.p. 63°. ^{1}H-NMR (CD_3OD): 6.11 (*s*, 2 H); 2.53–2.50 (*m*, 2 H); 2.12 (*s*, 3 H); 1.52 (*sept.*, $J = 6.0$, 1 H); 1.37–1.33 (*m*, 2 H); 1.48–1.21 (*m*, 10 H); 1.18–1.12 (*m*, 2 H); 1.12–1.06 (*m*, 2 H); 0.92 (*d*, $J = 6.0$, 3 H); 0.87 (*d*, $J = 6.4$, 6 H); 0.85 (*d*, $J = 6.8$, 3 H). ^{13}C-NMR (CD_3OD): major isomer: 157.2 (2 C); 137.0; 114.7; 108.6 (2 C); 40.7; 38.72; 38.63; 38.62; 37.6; 37.5; 34.3; 34.1; 29.3; 26.1; 25.6; 23.3; 23.2; 21.5; 20.5; 20.3. HR-TOF-MS: 333.2779 ($[M-H]^-$, $[C_{22}H_{37}O_2-H]^-$; calc. 333.2794).

3-Methyl-2-(3,7,11-trimethyldodec)benzene-1,3-diol (**9**). Pale yellow solid. M.p. 40°. ^{1}H-NMR (CD_3OD): 6.12 (*d*, $J = 2.0$, 1 H); 6.10 (*d*, $J = 2.8$, 1 H); 2.53–2.50 (*m*, 2 H); 2.16 (*s*, 3 H); 1.52 (*sept*, $J = 6.0$, 1 H); 1.36–1.33 (*m*, 2 H); 1.48–1.21 (*m*, 10 H); 1.18–1.12 (*m*, 2 H); 1.12–1.06 (*m*, 2 H); 0.95 (*d*, $J = 6.4$, 3 H); 0.88 (*d*, $J = 6.0$, 6 H); 0.86 (*d*, $J = 6.0$, 3 H). ^{13}C-NMR (CD_3OD): major isomer: 157.1; 156.2; 138.7; 120.8; 109.5; 101.3; 40.7; 38.7; 38.6; 38.5; 37.9; 37.8; 34.5; 34.1; 29.3; 26.1; 25.6; 23.3; 23.2; 20.4; 20.3; 19.9. HR-TOF-MS: 333.2771 ($[M-H]^-$, $[C_{22}H_{37}O_2-H]^-$; calc. 333.2794).

Cytotoxicity Assay. The cytotoxicities of compounds **1**–**9** against human lung cancer cells A549, mouse melanoma B16F1, and human melanoma SK-Mel-2 cell lines were determined by a colorimetric sulforhodamine B (SRB) assay [9]. Briefly, cancer cell lines were placed at a density of 5×10^4, 2×10^4, and 1×10^5 cells/ml for A-549, B16F1, and SK-Mel-2, resp., in RPMI-1640 medium (100 µl) in a 96-well plate. After 24 h incubation at 37° under humidified 5% CO_2, serially-diluted test solns. (100 µl in RPMI media) were added to the wells and incubated for another 48 h. Cell viability was determined by the relative optical density of the bound SRB dye, compared to the control, which was measured at 520 nm in a microplate reader. The results were expressed as the concentration at which there was 50% inhibition (IC_{50}).

Free Radical Scavenging Assay. Radical scavenging activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [22]. Serially diluted solns. (20 µl) of test samples were added to an EtOH soln. (80 µl) of DPPH (59 µg/ml) in a 96-well plate. After incubation for 30 min with shaking at 24°, changes in absorbance were measured in a 96-well microplate reader at 517 nm. The concentration required for a 50% decrease in the absorbance of a control soln. of DPPH was expressed as IC_{50}.

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