Chemical Constituents from *Aphanamixis grandifolia*

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(23*E*)-25-Methylcycloart-23-en-3β-ol (1), a cycloartane-type triterpenoid featuring an unusual skeleton of 31 carbon atoms, (17*E*)-cycloart-17,26-dien-3β-ol (2), a new cycloartane-type triterpenoid, and the other two new compounds 4*R*-hydroxy-4-(9*S*-hydroxy-12-methylhexan-6-yl)-3-methylcyclopent-2-enone (6) and 7-hydroxy-5-(2′-hydroxy-4′,5′-dimethoxyphenyl)-2-methoxy-6-methyl-1,4-naphthoquinone (7), together with three known cycloart-3β-ol triterpenoids (3—5) were isolated from aerial parts of *Aphanamixis grandifolia*. Their structures were elucidated by spectroscopic analysis, and that of 1 was confirmed by single-crystal X-ray diffraction. The absolute configuration of two carbon stereocenters of compound 6 was determined to be 4*R*,9*S* by means of circular dichroism (CD) and auxiliary chiral α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) derivatives, respectively. The compound 3 exhibited significant cytotoxicities against HL-60, Hep G2 and HeLa, and compounds 1, 2, 5, 6 and 7 exhibited modest cytotoxicities. No activity of compound 4 could be due to the absence of the hydroxyl group at C-24.

Key words *Aphanamixis grandifolia*; cycloartane-type triterpenoid; cyclopent-2-enone; 1,4-naphthoquinone; cytotoxicity

The genus *Aphanamixis* (Meliaceae) comprising about 5 species and varieties is mainly distributed in China, India and Indonesia. *Aphanamixis grandifolia*, an arbor tree mainly growing in southern China, is a medicinal plant employed as an astringent for spleen, liver, tumors, abdominal diseases, and applied in treatment of rheumatism in southeast Asia.[1)] It has not been chemically studied previously. In our current study, four new compounds (**1**, **2**, **6**, **7**) and three known compounds (**3**—**5**) were isolated from aerial parts of *Aphanamixis grandifolia* collected from Hainan Province, People's Republic of China. The structures of the new compounds were characterized using spectroscopic methods, and the structure of compound **1** was confirmed by single-crystal X-ray diffraction analysis. The known compounds were elucidated by comparing their spectroscopic data with those reported in the literature. Their structures were identified as (23*E*)-25-methylcycloart-23-en-3β-ol (**1**), a novel triterpenoid possessing a C_{31} skeleton, (17*E*)-cycloart-17,26-dien-3β-ol (**2**), (23*E*)-cycloart-23-en-3β,25-diol (**3**),[2)] (23*E*)-25-methoxycycloart-23-en-3β-ol (**4**),[2)] cycloart-3β,25-diol (**5**),[3)] 4*R*-hydroxy-4-(9*S*-hydroxy-12-methylhexan-6-yl)-3-methylcyclopent-2-enone (**6**), and 7-hydroxy-5-(2′-hydroxy-4′,5′-dimethoxyphenyl)-2-methoxy-6-methyl-1,4-naphthoquinone (**7**). In addition, the cytotoxicities of the compounds were evaluated against selected cancer cell lines, including human leukaemia cell (HL-60), human hepatoma cell (Hep G2), and human cervical carcinoma cell (HeLa) lines. The compound **3** was active (HL60 cell, IC_{50}=5.97 μg/ml; Hep G2, IC_{50}=20.85 μg/ml; HeLa, IC_{50}=24.89 μg/ml), compounds **1**, **2**, **5**, **6** and **7** exhibited modest cytotoxicities. Herein we report the isolation, structural elucidation, and biological activities of these compounds.

Results and Discussion

The ethanolic extract of the aerial parts of *Aphanamixis grandifolia* was concentrated under vacuum and then subjected repeatedly to column chromatography over silica gel, Sephadex LH-20, and MCI yield compounds **1**—**7** (Chart 1).

The ^{1}H-NMR spectra of compounds **1**—**5** indicated the presence of a cycloartane skeleton with 3β-ol group on C-3.[2)] This fact was evidenced by the typical H-19 doublets at δ_H 0.33 (J=3.9 Hz) and 0.55 (J=3.9 Hz), and the oxygenated methine near at δ_H 3.30 (m). Comparison of the ^{13}C-NMR spectra of **1**—**5** with those of related cycloart-3β-ol compounds in the literature,[2)] the methylene of C-19 at δ_C 30.0 and the methine of C-3 at δ_C 78.8, confirmed that these were all 3β-hydroxycycloartanes.

Compound **1** was obtained as a colorless crystal. The high resolution-electronspray ionization-mass spectra (HR-ESI-MS) displayed a molecular ion peak at m/z 441.4100 $[M+H]^+$ (Calcd for $C_{31}H_{53}O$, 441.4096) consistent with a molecular formula of $C_{31}H_{52}O$, requiring seven degrees of unsaturation, which was confirmed by ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) analysis. The ^{13}C-NMR spectrum indicated 31 resonances ascribed to seven tertiary methyl, one secondary methyl, ten methylene, seven methine (one oxygenated and two sp^2 hybridized), and six quarternary carbon atoms, which indicated that compound **1** was a pentacyclic triterpenoid possessing a double bond (δ_C 125.6, 139.4) and one oxygen-bearing functional group. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of **3**, a cycloartane-type triterpenoid skeleton. The MS and ^{1}H-NMR data revealed that **1** contained one more methyl singlet (δ_H 1.33, s; δ_C 29.9) than **3**. In addition, the significant

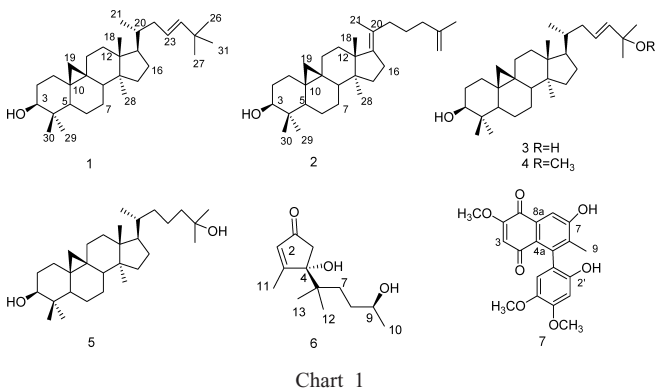

Chart 1

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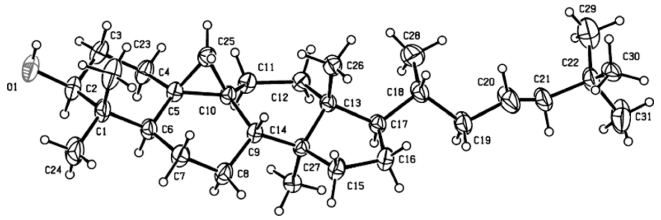

Fig. 1. Single-Crystal X-Ray Structure of **1**

difference in the ^{13}C-NMR spectra of **1** and **3** was that one quarternary carbon carbon at δ_C 40.5 (C) appeared in **1** instead of a downfield oxygenated carbon at δ_C 70.8 (C) in **3**. The methyl of C-31 should be linked at C-25, due to the evident that the H-26 and H-27 (δ_H 1.33, s) were also tertiary methyl. In the ^{1}H-NMR spectrum two proton signals assigned to a 1,2-disubstituted double bond appeared at δ_H 5.60 (2H, br s). It was also supported by the ^{13}C-NMR spectrum, where signals were observed at δ_C 125.6 and 139.3. The olefinic proton signals of a *trans*-double bond were usually overlapped in $CDCl_3$ solution in cycloart-23-en-25-ol derivatives, which was verified by Takahashi *et al.*[4] Compared with the reported data,[2,4] the stereochemistry of the double bond should be *E*. The structure of **1** was further confirmed by the single-crystal X-ray diffraction (Fig. 1). Compound **1** was established to be (23*E*)-25-methylcycloart-23-en-3β-ol. To our knowledge, the cycloartane triterpenoids were widely distributed in natural sources, but the cycloartane derivatives with the skeleton of 31 carbon atoms was rare.

Compound **2** was an optically active, $[\alpha]_D^{20}$ +11 (c=0.2, $CHCl_3$), amorphous solid. The molecular formula was determined as $C_{30}H_{48}O$ by HR-ESI-MS (*m*/*z* 425.3775 $[M+H]^+$, Calcd for 425.3778) associated with NMR data (Table 1) and indicated seven degrees of unsaturation. The ^{13}C-NMR and DEPT spectra showed 30 carbon resonances including six tertiary methyl (δ_C 13.9, 18.2, 20.0, 25.4, 29.4, 30.4), twelve methylene (one olefinic at δ_C 114.0), three methine (one oxymethine at δ_C 78.8), and seven quaternary carbons (three olefinic at δ_C 129.7, 134.0, 142.3). These data, when they coupled with the information from the ^{1}H-NMR spectrum [six tertiary methyl (δ_H 0.81, 0.85, 0.96, 0.96, 1.25, 1.31), two olefinic protons at δ_H 5.59 (br s), 5.60 (br s), and one oxymethine proton at δ_H 3.15 (m)], indicated that compound **2** was based on a cycloartane triterpenoid skeleton. The ^{1}H-NMR spectrum showed two olefinic signals at δ_H 5.59 (br s) and 5.60 (br s), and the chemical shifts corresponded to two double bonds with three quaternary carbons at δ_C 142.3, 134.0, 129.7 and a sp^2 methylene carbon at δ_C 114.0 in the ^{13}C-NMR and DEPT experiments. OH-3 was assigned β-orientations because of the nuclear overhauser effect (NOE) correlation between H-3 (δ_H 3.28) and H-5b (δ_H 1.68). A computer modeled 3D structure of **2** was generated by using MM2 force field calculations for energy minimization with the molecular modeling program Chem3D Ultra 9.0. The relative stereochemistry and the conformation of **2** assigned by NOE spectrums were compatible with those of **2** offered by computer modeling, in which the close contacts of atoms calculated in space were consistent with the NOE correlations. Therefore, and in view of biogenetic consideration, the structure of **2** was identified as (17*E*)-cycloart-17,26-dien-3β-ol.

Table 1. NMR Data of Compounds **1**—**3** in $CDCl_3$ (J in Hz)

Carbon	**1**		**2**		**3**
	δ_C	δ_H(J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	32.0	1.48 (m), 1.52 (m)	32.7	1.39 (m), 1.48 (m)	32.0
2	30.4	1.42 (m), 1.57 (m)	32.0	1.43 (m), 1.55 (m)	30.4
3	78.8	3.30 (m)	78.8	3.28 (m)	78.8
4	40.5		39.7		40.5
5	47.1	1.70 (m)	47.1	1.68 (m)	47.1
6	21.1	1.96 (m), 1.93 (m)	21.1	1.98 (m), 1.92 (m)	21.1
7	26.0	1.36 (m)	26.1	1.29 (m)	26.0
8	47.9	1.81 (m)	48.0	1.85 (m)	47.9
9	20.0		18.8		20.0
10	26.1		26.1		26.1
11	26.4	1.26 (m), 1.60 (m)	26.4	1.35 (m), 1.58 (m)	26.4
12	32.8	0.90 (m), 1.64 (m)	35.6	0.91 (m), 1.63 (m)	32.8
13	45.3		45.3		45.3
14	48.8		48.8		48.8
15	35.5	1.36 (m), 1.24 (m)	36.8	1.35 (m), 1.26 (m)	35.6
16	28.0	1.28 (m)	28.2	1.29 (m)	28.1
17	52.0	1.28 (m)	134.0		52.0
18	18.1	0.98 (s)	18.1	0.92 (m)	18.0
19	30.0	0.33 (d, J=3.9), 0.56 (d, J=3.9)	29.9	0.33 (d, J=3.6), 0.55 (d, J=3.6)	29.9
20	36.4	1.90 (m)	129.7		36.4
21	18.3	0.86 (d, J=6.5)	30.4	1.31 (br s)	18.2
22	39.0	2.16 (m)	52.2	2.03 (m)	39.0
23	125.6	5.60 (br s)	18.2	1.31 (m)	125.6
24	139.4	5.60 (br s)	40.5	1.98 (m)	139.3
25	40.5		142.3		70.8
26	30.0	1.33 (s)	114.0	5.59 (br s), 5.60 (br s)	29.9
27	30.0	1.33 (s)	29.7	1.25 (s)	29.9
28	19.3	0.85 (s)	20.0	0.81 (s)	19.2
29	25.4	0.88 (s)	25.4	0.96 (s)	25.4
30	14.0	0.88 (s)	13.9	0.96 (s)	13.9
31	29.9	1.33 (s)			

The double bond located at 17 and 20, which was rare in natural resources and will enter into the architectural diversity of the cycloartane family.

Compound **6** displayed a molecular ion peak at *m*/*z* 227.1649 (Calcd for 227.1647) in the HR-electron ionization (EI)-MS corresponding to the molecular formula of $C_{13}H_{22}O_3$. The IR absorption bands at 3392 and 1712 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{13}C-NMR data of compound **6** resolved 13 resonances attributable to four methyl groups, three methylene, two methane (one oxygenated and one olefinic), and four quaternary carbons (α,β-unsaturated carbonyl and one oxygenated). The ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectroscopic analyses of **6** revealed its planar structure and led to unambiguous assignments of the NMR data (Fig. 2). The absolute configuration of C-4 in **6** was determined from the circular dichroism (CD) spectrum (Fig. 3). On the basis of the octant rule for cyclopentanones and related reports, the positive Cotton effect at 330 nm ($\Delta\varepsilon_{max}$=+0.76) for $n\rightarrow\pi^*$ and the negative Cotton effect at 246 nm ($\Delta\varepsilon_{max}$=−5.61) for $\pi\rightarrow\pi^*$ indicated that the configuration at C-4 was as de-

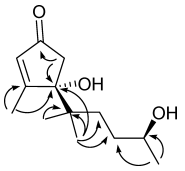

Fig. 2. Key HMBC Correlations of Compound **6**

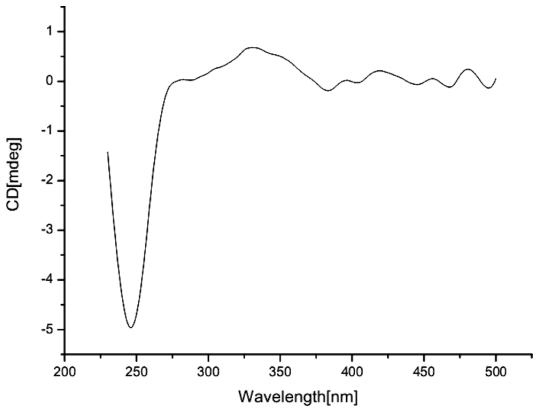

Fig. 3. CD Spectrum of Compound **6**

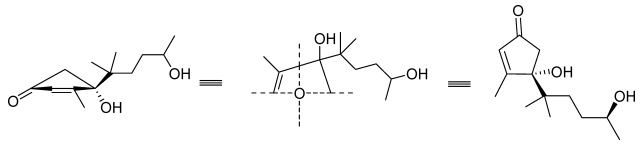

Fig. 4. Absolute Configuration of C-4 in Compound **6**

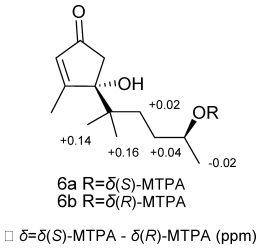

Fig. 5. ^{1}H-NMR Chemical Shift Differences [δ(*S*)-MTPA$-\delta$(*R*)-MTPA] of the MTPA Esters for Compound **6**

picted in Fig. 4. Therefore, the absolute configuration at the chiral centers of C-4 was 4*R*. Compound **6** was treated with (*R*)- and (*S*)- α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA)-Cl, and the (*S*)- and (*R*)-MTPA esters at C-9 of **6** (**6a**, **6b**) were obtained, respectively. Comparison of the ^{1}H-NMR chemical shifts for **6a** and **6b** (Δ values shown in Fig. 5) led to the assignment of the *S*-configuration at C-9 in **6**. Hence, the structure of compound **6** was elucidated as 4*R*-hydroxy-4-(9*S*-hydroxy-12-methylhexan-6-yl)-3-methylcyclopent-2-enone.

Compound **7** exhibited a molecular ion peak at *m*/*z* 371.1136 (Calcd for 371.1131) in the HR-EI-MS indicating a molecular formula of $C_{20}H_{18}O_7$. The IR spectrum of **7** showed absorptions at 3311 (hydroxy), 2923 (α,β-unsaturated carbonyl) and 1671 cm^{-1} (carbonyl). The UV spectrum showed absorption at 248, 286, and 318 nm, resembling those of naphthoquinone derivatives, which was confirmed by the positive result in Magnesium Acetate test. The ^{13}C-NMR and DEPT spectrum revealed that **7** contains fourteen olefinic carbons, four methyl (three oxygenated) and two carbonyl groups. A comparison of its NMR data with those of 2-methoxy-5-(3,4-dimethoxyphenyl)-6-ethoxycarbonyl-7-hydroxy-1,4-naphthoquinone,[5] indicated that **7** was an analogue of 1,4-naphthoquinones. The structure was determined by analysis of the HMBC data of **7** (Fig. 6). All methoxyl resonances were assigned by NOE correlations (Fig. 7). Finally, the structure of **7** was established as 7-hydroxy-5-(2′-hydroxy-4′,5′-dimethoxyphenyl)-2-methoxy-6-methyl-1,4-naphthoquinone. The 1,4-naphthoquinone derivatives including vitamin K, plumbagin (PL), juglone, and shikonin are well known to possess various pharmacologic properties.[6] To the best of our knowledge, the skeleton of 5-aryl-6-methyl-1,4-naphthoquinone was synthesized artificially as a synthetic product, and rarely found in nature.

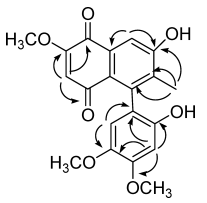

Fig. 6. Key HMBC Correlations of Compound **7**

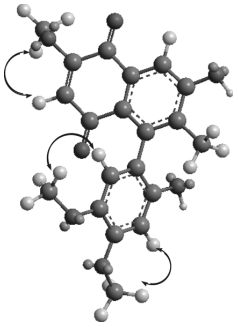

Fig. 7. Key NOE Correlations of Compound **7**

In *in vitro* cytotoxic assays using the sulforhodamine B (SRB) method[7] against several human cancer cell lines including human leukemia (HL-60), human hepatoma (Hep G2), human cervical carcinoma (HeLa), compound **3** showed significant cytotoxicities against HL-60 (IC_{50}=5.97 μg/ml), Hep G2 (IC_{50}=20.85 μg/ml) and HeLa (IC_{50}=24.89 μg/ml), compounds **1**, **2**, **5**, **6** and **7** exhibited modest cytotoxicity (Table 4). Comparing with compounds **1**, **2**, **3** and **5**, the lack of activity of compound **4** could be caused by the absence of the hydroxyl group on C-24, in spite of the existence of the cycloartane skeleton.

Experimental

General Experimental Procedures IR spectra were recorded on a Nicolet 170SX Fourier transform-infrared (FT-IR) instrument using KBr discs over the range 400—4000 cm^{-1}. UV detections were measured on a Shimadzu UV-260 spectrophotometer. NMR spectra were obtained on a Bruker AM-400 and a Varian Mercury-300/400BB NMR spectrometer with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. HR-ESI-MS were obtained on a Bruker Daltonics APEX-II 47e spectrometer. EI-MS data were obtained on an HP5988AGCMS spectrometer. HR-ESI-MS data were measured on a Bruker Daltonics APEX II 47e spectrometer. The X-ray crystallographic data were collected on a Bruker Smart CCD diffractometer using graphic-monochro-mate Mo$K\alpha$ radiation. Analytical and preparative thin-layer chromatographys (TLC) were performed on silica gel plates (GF_{254}

Table 2. NMR Data of Compound **6** in $CDCl_3$ (J in Hz)

	δ_C	δ_H (J in Hz)
1	198.1	
2	126.0	5.85 (s)
3	168.3	
4	77.8	
5	34.8	2.2 (d, J=18.0), 2.5 (d, J=18.0)
6	41.7	
7	49	1.85 (m), 1.95 (m)
8	33.5	1.54 (m), 1.62 (m)
9	68.7	3.79 (m)
10	21.8	1.23 (d, J=6.3)
11	24.2	2.05 (s)
12	23.8	1.09 (s)
13	23.7	1.05 (s)

Table 3. NMR Data of Compound **7** in $CDCl_3$ (J in Hz)

	δ_C	δ_H
1	185.2	
2	160.7	
3	125.5	6.14 (s)
4	181.0	
4a	128.1	
5	123.8	
6	128.5	
7	158.6	
8	108.7	7.99 (s)
8a	124.4	
9	16.0	2.66 (s)
1′	114.8	
2′	153.7	
3′	94.5	7.11 (s)
4′	146.0	
5′	151.8	
6′	108.5	8.80 (s)
2-OMe	56.4[a)]	3.96 (s)
4′-OMe	56.6[a)]	4.02 (s)
5′-OMe	56.5[a)]	4.09 (s)

a) Signals within same column may be interchangeable.

10—40 μm, Qingdao Marine Chemical Factory). Analytical TLC was provided to follow the separation and check the purity of isolated compounds. Spots on the plates were observed under UV light at 254 nm and visualized by spraying them with 5% H_2SO_4 in EtOH (v/v), followed by heating. Column chromatography (CC) was performed on silica gel (200—300 mesh, Qingdao Marine Chemical Factory). The spots were detected on TLC by heating to 90 °C after spraying with 0.5% Magnesium Acetate in CH_3OH (v/v) in the Magnesium Acetate test.

Plant Material The aerial parts of *Aphanamixis grandifolia* were collected in Tuncang County of Hainan Province, China, July 2007, and identified by Qiongxin Zhong of Hainan Normal University. The voucher specimen (No. 2007006) was deposited in the State Key Laboratory of Applied Organic Chemistry, Lanzhou University, China.

Extraction and Isolation The air-dried, powdered aerial parts of *A. grandifolia* (6.94 kg), were extracted with a mixture of EtOH at room temperature. After evaporation of the solvent *in vacuo*, the residue (498.1 g) was fractionated on a silica gel column with a gradient mixture of petroleum ether–Me_2CO and finally MeOH to give eight fractions (Fr. A—Fr. H). Fr. D was extensively separated over the columns of silica gel (PE : AcOEt, 20 : 1, 10 : 1; $CHCl_3$: AcOEt, 60 : 1) to give **1** (8 mg), **2** (6 mg) and **4** (3 mg). Fr. E was subjected to by chromatography on MCI (EtOH : H_2O, 50%, 80%, 100%) and repeatly separated on silica gel columns, and then purified by chromatography on a Sephadex LH-20 column ($CHCl_3$: MeOH, 1 : 1) to afford **3** (32 mg), **5** (1 mg), **6** (6 mg), **7** (9 mg).

(23*E*)-25-Methylcycloart-23-en-3β-ol (**1**): Colorless crystal, mp 248—250 °C. $[\alpha]_D^{20}$ +34 (c=0.2, $CHCl_3$); IR (KBr) ν_{max} 3395, 2959, 2855, 1711,1458, 1383, 1047 cm^{-1}; ^{1}H- and ^{13}C-NMR data (see Table 1); EI-MS (pos.) m/z 440 $[M]^+$; HR-ESI-MS (pos.) m/z 441.4100 $[M+H]^+$ (Calcd for $C_{31}H_{53}O$, 441.4096).

(17*E*)-Cycloart-17,26-dien-3β-ol (**2**): Amorphous solid, $[\alpha]_D^{20}$ +11 (c= 0.2, $CHCl_3$); IR (KBr) ν_{max} 3337, 2923, 2858, 1709, 1458, 1379, 1097, 1026 cm^{-1}; ^{1}H- and ^{13}C-NMR data (see Table 1); EI-MS (pos.) m/z 424 $[M]^+$; HR-ESI-MS (pos.) m/z 425.3775 $[M+H]^+$ (Calcd for $C_{30}H_{49}O$, 425.3778).

4*R*-Hydroxy-4-(9*S*-hydroxy-12-methylhexan-6-yl)-3-methylcyclopent-2-enone (**6**): Amorphous solid, $[\alpha]_D^{20}$ −12 (c=0.2, $CHCl_3$); IR (KBr) ν_{max} 3392, 2964, 2875, 1711, 1652, 1456, 1379, 1051 cm^{-1}; UV 246, 252, 258 and 330 nm; ^{1}H- and ^{13}C-NMR data (see Table 2); EI-MS (pos.) m/z 226 $[M]^+$; HR-ESI-MS (pos.) m/z 227.1649 $[M+H]^+$ (Calcd for $C_{13}H_{23}O_3$, 227.1647).

7-Hydroxy-5-(2′-hydroxy-4′,5′-dimethoxyphenyl)-2-methoxy-6-methyl-1,4-naphthoquinone (**7**): Red solid, $[\alpha]_D^{20}$ −4 (c=0.2, $CHCl_3$); IR (KBr) ν_{max} 3311, 2923, 2853, 1671, 1461, 1379, 1301, 1073 cm^{-1}; UV 248, 286, and 318 nm; ^{1}H- and ^{13}C-NMR data (see Table 3); EI-MS (pos.) m/z 370 $[M]^+$; HR-ESI-MS (pos.) m/z 371.1136 $[M+H]^+$ (Calcd for $C_{20}H_{19}O_7$, 371.1131).

(*R*)- and (*S*)-MTPA Derivatives of 6 To a solution of compound **6** (2.0 mg, 8.9×10^{-3} mmol) in (dimethylamino)pyridine (5.3 mg, 43.2×10^{-3} mmol) and triethylamine (4.5 μl, 31.5×10^{-3} mmol) at room temperature was added (*S*)-MTPA-Cl (4.02 μl, 22.1×10^{-3} mmol), and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was worked up by adding 2 ml of water. Further purification was performed with the columns of silica gel with petroleum ether–EtOAc, 5 : 1, to give the (*R*)-MTPA ester **6b** (1.1 mg) as a colorless gum. The (*S*)-MTPA ester **6a** (1.2 mg) was prepared in a similar manner.

X-Ray Crystal Structure Analysis of 1[8)] A crystal of **1** with dimensions 0.35×0.29×0.24 mm was selected for X-ray analysis. Structure analysis was performed using the SHELEXTL-97 program on a PC. Data were collected over a hemisphere of reciprocal space, by a combination of three sets of exposures. The compound crystallized in the monoclinic space group $C2$, with a=33.814(2) Å, b=7.4041(5) Å, c=11.1212(8) Å, β=98.276(6)°, V=2755.4(3) Å^3, Z=4, D_{calc}=1.062 g/cm^3, λ=0.71073 Å, μ (Mo$K\alpha$)= 0.061 mm^{-1}, F(000)=984, and T=296(2) K. The SMART program was used to make data corrections. A total of 7899 reflections, collected in the range 1.22°≤θ≤26.00°, yielded 4925 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on $F2$ values for 4925$I>2\sigma(I)$. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R=0.0770, R_w=0.1898 with goodness-of-fit=1.045. Scattering factors were taken from 'International Tables for X-ray Crystallography.'[9)]

Biological Activity The cytotoxicities of compounds **1**—**7** toward human leukaemia cell (HL-60), human hepatoma cell (Hep G2) and human cervical carcinoma cell (HeLa) lines were determined in 96-well microtiter plates by the sulforhodamine B method described by Skehan *et al.*[7)] Briefly, exponentially growing cancer cells were harvested and seeded in 96-well plates with the final volume 100 μl containing 4×10^3 cells per well. After 24 h incubation, cells were treated with various concentrations of these compounds for 48 h. The cultures were fixed at 4 °C for 1 h by addition of ice-cold 50% trichloroacetic acid (TCA) to give a final concentration of 10%. Fixed cells were rinsed 5 times with deionized water and stained for 10 min with 0.4% sulforhodamine B dissolved in 1% acetic acid. The wells were washed 5 times with 1% acetic acid and left to dry overnight. The absorbed sulforhodamine B was dissolved in 150 μl unbuffered 1% Tris base [tris(hydroxymethyl)aminomethane] solution in water (pH 10.5). The absorbency of

Table 4. Cytotoxicity Assay for Isolated Compounds **1**—**7**

	IC_{50} (μg/ml)		
	HL60 cell	Hep G2 cell	HeLa cell
Mitomycin	0.56±0.17	1.85±0.53	1.11±0.64
1	69.24±6.75	82.63±7.52	NE
2	36.70±6.10	50.54±6.35	35.83±4.03
3	5.97±1.13	20.85±3.80	24.89±4.81
4	NE	NE	NE
5	39.16±5.52	55.69±3.43	39.96±3.80
6	51.12±11.23	85.44±0.80	109.66±11.83
7	21.44±4.83	86.75±9.36	50.87±6.72

extracted sulforhodamine B at 515 nm was measured on a microplate reader (Bio-Rad). The experiments were carried out in triplicate. Each run entailed 5—6 concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

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