Phenolic Compounds from the Branches of *Eucalyptus maideni*

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Three new phenolic compounds, eucalmaidin F (**1**), (3*S*)-5-guaiacyl-3-hydroxypentanoic acid (**2**), and 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isobutylchromone (**3**), were isolated from the branches of *E. maideni*, together with 30 known compounds, including four phenylpropanoids, three lignans, four phloroglucinol glucosides, five dihydroflavonoids, seven simple phenolic compounds, six terpenoids, and glycerol. The new structures were established by spectroscopic studies (MS, and 1D- and 2D-NMR), chemical degradation, and modified *Mosher*'s method. Compounds **3**, guaiacylglycerol, 3-hydroxy-1-(4-hydroxyphenyl)propan-1-one, caffeic acid, (2*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid, (7′*S*,8*R*,8′*R*)-lyoniresinol, (+)-lyoresinol 3α-*O*-α-L-rhamnopyranoside, garcimangosone, phlorocetophenone 2′-glucopyranoside, (+)-taxifolin 3α-*O*-α-L-rhamnopyranoside, (+)-aromadendrin, (+)-taxifolin, resveratrol, piceatannol, 3,4,5-trihydroxyphenol. Tachiaside, gallic acid, macrocapals A und G, and oleuropeic acid were evaluated for their cytotoxicities against five human cancer cell lines. Resveratrol, piceatannol, gallic acid, and macrocapal G exhibited moderate inhibitory effects on human myeloid heukemia HL-60 cell, with IC_{50} values of 22.05, 22.05, 7.75, and 31.93 μM, respectively; and only macrocapal G showed inhibitory effect on hepatocellular carcinoma SMMC-7721 cell, with an IC_{50} value of 26.75 μM.

Introduction. – The genus *Eucalyptus* (Myrtaceae), mainly occurring in the tropical and subtropical areas of the world, is known to be a rich source of bioactive secondary metabolites. A series of terpenoids, tannins, flavonoids, and phloroglucinol derivatives with antiviral and antibacterial effects have been reported [1][2]. In our previous works, five new (+)-oleuropeic acid derivatives [3] and five new phloroglucinol glycosides [4] were identified, respectively, from the fresh leaves and fresh fruits of *Eucalyptus maideni* F. MUELL., a tall timber tree growing widely in the southern parts of China. To further study the chemical constituents of *Eucalyptus* trees and to search for bioactive phenolics, the investigation on the air-dried branches of this species was carried out. This led to the isolation of three new phenolic compounds, eucalmaidin F (**1**), (3*S*)-5-guaiacyl-3-hydroxypentanoic acid (**2**), and 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isobutylchromone (**3**; *Fig. 1*), together with 30 known compounds, including four phenylpropanoids, three lignans, four phloroglucinol glucosides, four dihydroflavonoids, seven other phenolic compounds, six terpenoids, and glycerol. Here, we report the isolation and structure elucidation of the new compounds. In addition, several compounds were evaluated for their cytotoxicity against five human cancer cell lines.

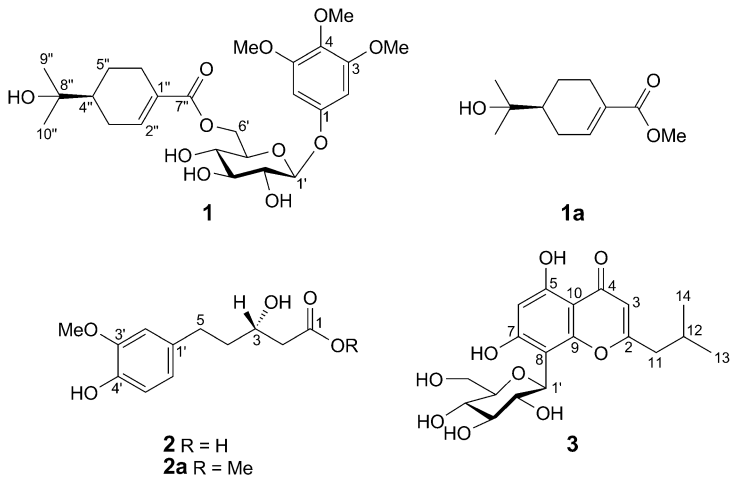


Fig. 1. *New compounds isolated from the branches of* Eucalyptus maideni

Results and Discussion. – The 80% aqueous acetone extract of the branches of *E. maideni* was partitioned between AcOEt and H_2O. The AcOEt fraction was subjected to various column chromatographies (CC) and semipreparative HPLC to yield 33 compounds. The known compounds were determined as guaiacylglycerol [5], 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one [6], caffeic acid, (2*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid [7], (7′*S*,8*R*,8′*R*)-lyoniresinol [8], (+)-lyoniresinol 3α-*O*-α-L-rhamnopyranoside [9], (+)-lyoniresinol 3α-*O*-β-D-xylopyranoside [10], garcimangosone [11], phloracetophenone 2′-glucopyranoside [12], eucalmainosides D and E [4], (+)-taxifolin 3-*O*-α-L-rhamnopyranoside [13], engelitin [14], (+)-aromadendrin [14], eriodictyol [14], (+)-taxifolin [14], (+)-catechin [7], resveratrol [15], piceatannol [16], 3,4,5-trihydroxyphenol [17], tachioside, gallic acid, 3-*O*-methylellagic acid 3′-*O*-α-L-rhamnopyranoside [4], macrocapals A and G [18], (±)-oleuropeic acid, β-sitosterol [7], icariside B_4 [19], eucalmaidin A [3], and glycerol, on the basis of detailed spectroscopic analyses, together with the comparison of their spectroscopic and physical data with those in the literature. (+)-Lyoniresinol 3α-*O*-α-L-rhamnopyranoside, (+)-lyoniresinol 3α-*O*-β-D-rhamnopyranoside, garcimangosone, and phloroacetophenone 2′-glucopyranoside were reported from the genus *Eucalyptus* for the first time.

Compound **1** was obtained as an amorphous powder. Its high-resolution (HR) ESI-MS displayed a $[M+Cl]^-$ ion peak at *m*/*z* 547.1954 (calc. for $C_{25}H_{36}ClO_{11}^-$, 547.1946), indicating the molecular formula $C_{25}H_{36}O_{11}$. The ^{1}H- and ^{13}C-NMR spectra showed one 2-H *singlet* (δ(H) 6.40 (*s*)) and signals of six symmetric aromatic C-atoms at δ(C) 155.6 (C), 154.8 (2 C), 135.0 (C), and 96.5 (2 CH), arising from a symmetrically substituted phloroglucinol moiety, a set of signals characteristic of a β-D-glucopyranosyl moiety (anomeric H-atom signal at δ(H) 4.85 (*d*, *J* = 7.7)), as well as signals of three MeO groups (δ(H) 3.79 (*s*, 6 H), 3.70 (*s*, 3 H)). Besides, the ^{13}C-NMR and DEPT spectra showed ten C-atom signals attributed to one COO group (δ(C) 168.7), one

trisubstituted C=C bond (δ(C) 141.5, 131.1), two Me groups (δ(C) 27.1, 26.4), three CH_2 groups (δ(C) 28.5, 26.3, 24.4), one CH group (δ(C) 45.6), and one O-bearing quaternary C-atom (δ(C) 72.8), arising from an oleuropeic acid unit. These data were similar to those of cypellocarpin A, an oleuropeic acid derivative isolated from *E. cypellocarpa* [20]. The positions of the oleuropeoyl ester and glycosidic linkages in **1** were established by 2D-NMR experiments. In the HMBC spectrum of **1**, the glucosyl CH_2(6′) (δ(H) 4.52, 4.23–4.21) and H–C(1′) (δ(H) 4.85) correlated, respectively, with the oleuropeoyl carboxylic C-atom C(7″) (δ(C) 168.7) and C(1) (δ(C) 155.6) of the phloroglucinol unit. In addition, the glucosyl anomeric H-atom (δ(H) 4.85) showed ROESY correlation with the phloroglucinol aromatic H-atoms (δ(H) 6.40), confirming the glycosidic linkage at C(1). Methanolysis of **1** with MeONa in MeOH afforded the (−)-oleuropeic acid methyl ester ($[\alpha]_D = -32.9$ ($CHCl_3$)) [21]. Based on the above evidence, the structure of **1** was determined to be (−)-3,4,5-trimethoxyphenol *O*-(6-*O*-oleuropeoyl)-β-D-glucopyranoside[1]) and named eucalmaidin F.

Compound **2** was obtained as a pale amorphous powder. The molecular formula $C_{12}H_{15}O_5$ was elucidated from the HR-ESI-MS (*m/z* 239.0915 ($[M-H]^-$, calc. 239.0919)). The ^{13}C-NMR spectrum of **2** revelead the presence of a COO group (δ(C) 179.7), six aromatic C-atoms (δ(C) 148.8 (C), 145.4 (C), 135.2 (C), 121.8 (CH), 116.1 (CH), and 113.2 (CH)); an O-bearing CH group (δ(H) 69.4), a MeO group (δ(H) 56.3), and three CH_2 group (δ(C) 44.7, 42.7, 32.5). In the ^{1}H-NMR spectrum, signals of three aromatic H-atoms (δ(H) 6.78 (*d*, $J = 1.6$), 6.69 (*d*, $J = 8.0$), and 6.63 (*dd*, $J = 8.0$, 1.6)), arising from a 1,3,4-trisubstitued benzene ring, of a MeO H-atom (δ(H) 3.82 (*s*)), and several aliphatic H-atom signals were observed. The ROESY correlation between the MeO H-atom (δ(H) 3.82) and H–C(2′) (δ(H) 6.78) indicated the location of the MeO group as C(3′), and the OH group was, accordingly, at C(4′) of the benzene ring. From the HSQC and ^{1}H,^{1}H-COSY spectra of **2**, a partial structure of –CH_2–CH(OH)–CH_2–CH_2– from C(2) to C(5) could be elucidated. In the HMBC spectrum of **2**, correlations of the signals of H–C(2) and H–C(3) with that of the carboxy C-atom, and of H–C(5) with those aromatic C-atoms C(1′), C(2′) and C(6′) positioned the partial structure –CH_2–CH(OH)–CH_2–CH_2– between the carboxy C-atom and the aromatic ring (*Fig. 2*), similar to 3-hydroxy-5-phenylpentanoic acid [19]. The absolute configuration of C(3) was determined by applying the modified *Mosher*'s method. Methylation of **2** with MeOH in AcCl afforded **2a**. Treatment of **2a** with (+)-(*R*)- and (−)-(*S*)-MTPA (MTPA = 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid) in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) provided mono ester derivatives, the $\Delta\delta$(H) (*S*–*R*) values (*Scheme*) established the (*S*) configuration at C(3) of **2**. Thus, compound **2** was determined to be (3*S*)-5-guaiacyl-3-hydroxypentanoic acid.

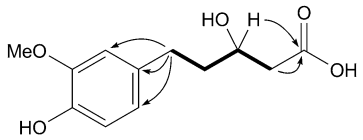
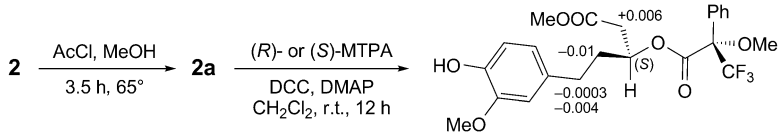


Fig. 2. *Key HMBC* (H → C) *and* ^{1}*H,*^{1}*H-COSY* (—) *correlations of compound* **2**

[1]) The absolute configuration of glucose was assumed to be D based on biogenetic considerations.

Scheme. *Determination of the Absolute Configuration of* **2** *by the MTPA Method*



Compound **3**, a pale yellow amorphous powder, had a molecular formula $C_{19}H_{23}O_9$, deduced from the HR-ESI-MS (*m/z* 395.1345 ($[M-H]^-$, calc. 395.1342)). The ^{1}H- and ^{13}C-NMR spectra of **3**, coupled with HSQC spectrum, exhibited two lower-field H-atom signals at δ(H) 6.06 and 6.23 (H–C(3) and H–C(6)); signals of a CH_2 group at δ(H) 2.58 and 2.50 (2*dd*, J=6.5, 13.9), of a CH group at δ(H) 2.21–2.23 (*m*) coupled with two Me signals at δ(H) 1.03 and 1.00 (3*d*, J=6.6), and a set of aliphatic H-atom signals arising from a β-glucopyranosyl moiety (anomeric H-atom signal at δ(H) 4.60 (*d*, J= 9.8)). These NMR features were similar to those of 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone [22]. However, instead of the signals of an i-Pr side chain at C(2) of the known compound, compound **3** exhibited the C-atom signals (δ(C) 44.1 (CH_2), 28.2 (CH), 22.8 (Me) and 22.5 (Me)) attributed to an isobutyl group. In the ^{13}C-NMR spectrum of **3**, resonances ascribable to chromone and glucosyl moieties were also observed. The HMBCs of the glucosyl anomeric H-atom with C(7) (δ(C) 165.1), C(8) (δ(C) 105.6), and C(9) (δ(C) 158.4) indicated the location of the β-*C*-glucopyranosyl unit as C(8) of the chromone skeleton. This was confirmed by comparison of the ^{13}C-NMR data in (D_6)DMSO of **3** (C(6) at δ(C) 98.2) with those of 8-β-*C*-(C(6) at δ(C) 98) and 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (C(8) at δ(C) 93) recorded in (D_6)DMSO [22]. Accordingly, the structure of **3** was determined as 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isobutylchromone.

Compound **3** and further 20 known compounds were evaluated for their cytotoxicities against five human cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method as reported in [23], with cisplatin as positive control. As a result, only compounds resveratrol, piceatannol, gallic acid, and macrocapal G exhibited moderate inhibitory effects on human myeloid heukemia HL-60 cell line with IC_{50} values of 22.05, 22.05, 7.75, and 31.93 μM, respectively, relative to 1.34 μM of cisplatin, and macrocapal G also showed inhibitory effect on hepatocellular carcinoma SMMC-7721 cell line with an IC_{50} value of 26.75 μM (cisplatin: 12.16 μM). All the tested compounds showed no activity to the breast cancer MCH-7, colon cancer SW480, and lung cancer A-549 cell lines, at a concentration of 40 μM.

Conclusions. – The branches of *Eucalyptus* species are rich in diverse phenolic compounds. Of them, oleuropeic acid derivatives may be evolved from terpineol, one of the major chemical constituents of *Eucalyptus* oil [24]. The isolated dihydroflavonoid, (+)-aromadendrin, and phenolic compounds, (+)-catechin, piceatannol, gallic acid, and 3-*O*-methylellagic acid 3′-*O*-α-L-rhamnopyranoside, also reported as the chemical constituents of *E. kino*, a dark exudate when the active cambium is injured [25], probably serve as the consistent preventive constituents of *Eucalyptus maideni*. The occurrence of macrocapals in the branches of *E. maideni* is very surprising, since

they are reported to be inducible second metabolites involved in host–pathogen interactions in the wounded wood of *E. globulus* and *E. nitens* [26]. Though some chemical constituents isolated from *Eucalyptus* plants displayed potential anti-tumor-promoting activities [20][27–29], only four compounds (see above) from this study showed moderate inhibitory effects on HL-60 and SMMC-7721 cell lines. The biological functions of these compounds need further studies.

Experimental Part

General. Column chromatography (CC): *MCI-gel CHP-20P* (75–150 μM; *Mitsubishi Chemical Co.*), *Sephadex LH-20* (25–100 μM; *Pharmacia Fine Chemical Co. Ltd.*), *Toyopearl HW-40F* (*TOSOH*, Japan), *RP-8* (40–63 μm; *Merck*), and silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Co. Ltd.*). TLC: SiO_2 plates; detection by anisaldehyde–H_2SO_4 reagent or 10% H_2SO_4 reagents, followed by heating. Semiprep. reversed-phase (RP) HPLC: *Waters 600* liquid chromatograph with a *Zorbax SB-C*$_{18}$ column. Optical rotations: *JASCO-20* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrometer; MeOH solns.; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker-AM-400* and *-DRX-500* spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG-Auto-Spec-3000* spectrometer with glycerol as matrix for FAB-MS; *API-QSTAR-Pulsar-1* spectrometer for ESI-MS and HR-ESI-MS; in *m*/*z*.

Plant Materials. The branches of *E. maideni* were collected in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China, during May 2007, and identified by Prof. *Xiao Cheng* (Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (KIB-ZL-200702) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resoures in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried branches of *E. maideni* (20 kg) were extracted with 80% aq. acetone at r.t. (3 × 30 l, each 1 week). After removal of org. solvents, the extracts were concentrated to a smaller volume (5 l) and partitioned with AcOEt (5 × 2 l) after filtration of the precipitate. The AcOEt fraction (220 g) was applied to CC (*Sephadex LH-20*, MeOH/H_2O 0:1–1:0) to afford seven fractions. *Fr. 1* (40 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 1:0:0→6:4:1) to yield *Frs. 1-1–1-5*. *Fr. 1-2* was applied to CC (*MCI-gel CHP20P*; MeOH/H_2O 4:6→7:3; *RP-8*; MeOH/H_2O 4:6→6:4) to yield *icariside B*$_4$ (7 mg). *Fr. 1-3* was subjected to CC (SiO_2; $CHCl_3$/MeOH, 95:5→8.5:1; *MCI-gel CHP20P*; MeOH/H_2O 2:8→1:0; and *RP-8*; MeOH/H_2O 4:6→7:3) to yield **1** (5 mg), *guaiacylglycerol* (51 mg), and *(±)-oleuropeic acid* (19 mg). *Fr. 1–4* was subjected to CC (SiO_2; $CHCl_3$/MeOH 9:1→7:3; *MCI-gel CHP20P*; MeOH/H_2O 0:1→1:1; and *Toyopearl HW-40F*; MeOH/H_2O 0:1→2:8) to yield *tachioside* (10 mg), *eucalmaidin A* (37 mg), and *glycerol* (68 mg). *Fr. 2* (8.2 g) was applied to CC (*MCI-gel CHP20P*; MeOH/H_2O 0:1→1:0) to yield four fractions, *Frs. 2-1–2-4*. *Fr. 2-2* was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0→9:1 and *Toyopearl HW-40F*; MeOH/H_2O 1:1→8:2) to yield *3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one* (5 mg). *Fr. 2-3* was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0→8.5:1.5; *MCI-gel CHP20P*; MeOH/H_2O 40→80%; *Toyopearl HW-40F*; MeOH/H_2O 1:1→8:2), followed by semiprep. HPLC (35% MeOH/H_2O) to yield **3** (14 mg), *(+)-lyoniresinol 3α-O-α-*L*-rhamnopyranoside* (9 mg), *(+)-lyoniresinol 3α-O-β-*D*-xylopyranoside* (4 mg), *eucalmainoside E* (4 mg), *(+)-taxifolin 3-O-α-*L*-rhamnopyranoside* (4 mg), and *(±)-oleuropeic acid* (235 mg). *Fr. 3* (18 g) was purified by CC (*MCI-gel CHP20P*; MeOH/H_2O 0→100%) to give three fractions, *Frs. 3-1–3-3*. *Fr. 3-1* was subjected to CC (SiO_2; $CHCl_3$/MeOH 98:2→8:2; *MCI-gel CHP20P*; MeOH/H_2O 2:8→7:3; and *Toyopearl HW-40F*; MeOH/H_2O 30→80%) to afford *3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one* (22 mg), *phloracetophenone 2′-glucopyranoside* (6 mg), and *eucalmainoside D* (10 mg). *Fr. 3-2* was chromatographed over *MCI-gel CHP20P* (MeOH/H_2O 20→80%), SiO_2 ($CHCl_3$/MeOH/H_2O 8.5:1.5:0.1→8:2:0.2) and *RP-8* (MeOH/H_2O 40→60%) to yield **2** (18 mg), *(7′*S*,8*R*,8′*R*)-lyoniresinol* (17 mg), and *garcimangasone* (69 mg). *Fr. 3-3* was subjected to CC (*MCI-gel CHP20P*; MeOH/H_2O 3:7→6:4) to yield *3-O-methylellagic acid 3-*O*-α-*L*-rhamnopyranoside* (16 mg). *Fr. 4* (36 g) was subjected to CC (*MCI-gel CHP20P*; MeOH/H_2O 0:1→1:1) to yield *gallic acid* (7 g). *Fr. 5* (29 g) was

subjected to CC (*MCI-gel CHP20P*; MeOH/H_2O 0:1→1:0; SiO_2; $CHCl_3$/MeOH 9:1→7.5:2.5; and *Toyopearl HW-40F*; MeOH/H_2O 1:1→1:0) to yield *caffeic acid* (300 mg), *(2E)-3-(4-hydroxyphenyl)-prop-2-enoic acid* (22 mg), *engelitin* (14 mg), and *(+)-aromadendrin* (22 mg). *Fr. 6* (33 g) was chromatographed over *MCI-gel CHP20P* (MeOH/H_2O 0→100%) to yield five fractions, *Frs. 6-1–6-5*. *Frs. 6-1* and *6-2* were subjected to CC (SiO_2; $CHCl_3$/MeOH 9:1→7:3; and *MCI-gel CHP20P*; MeOH/H_2O 4:6→8:2) to yield *(+)-catechin* (77 mg) and *resveratrol* (24 mg). *Fr. 6-3* was subjected to CC (SiO_2; $CHCl_3$/MeOH 9:1→7:3; and *Toyopearl HW-40F*; MeOH/H_2O 3:7→8:2) to yield *eriodictyol* (230 mg), and *(+)-taxifolin* (4 mg). *Frs. 6-4* and *6-5* were subjected to CC (*MCI-gel CHP20P*; MeOH/H_2O 7:3→1:0) to yield *macrocarpal A* (65 mg), *macrocarpal G* (50 mg), and *β-sitosterol* (6 mg). *Fr. 7* (52 g) was repeatedly subjected to *MCI-gel CHP20P* (70 to 100% MeOH/H_2O) to yield *piceattanol* (5 mg), and *3,4,5-trihydroxyphenol* (90 mg).

Eucalmaidin F (= *3,4,5-Trimethoxyphenyl 6-*O*-[[(4*S*)-4-(1-Hydroxy-1-methylethyl)cyclohex-1-en-1-yl]carbonyl]-β-*D*-glucopyranoside*; **1**). Pale amorphous powder. $[\alpha]_D^{15} = -45.4$ ($c = 0.2$, MeOH). UV (MeOH): 205 (4.65), 308 (3.38). IR: 3431, 2964, 2930, 1709, 1601, 1505, 1464, 1128, 1070. ^{1}H-NMR (500 MHz, CD_3OD): 6.96–6.95 (*m*, H–C(2″)); 6.40 (*s*, H–C(2/6)); 4.85 (*d*, *J* = 7.7, H–C(1′)); 4.52 (*dd*, *J* = 2.0, 12.0, H–C(6′)); 4.23–4.21 (*m*, H–C(6′)); 3.79 (*s*, MeO–C(3), MeO–C(5)); 3.78–3.68 (*m*, H–C(5′)); 3.70 (*s*, MeO–C(4)); 3.46 (*t*, *J* = 8.9, H–C(3′)); 3.42 (*t*, *J* = 7.7, H–C(2′)); 3.36 (*t*, *J* = 8.9, H–C(4′)); 2.46–2.43 (*m*, H–C(6″)); 2.31–2.30 (*m*, H–C(3″)); 2.10–2.08 (*m*, H–C(6″)); 2.00–1.97 (*m*, H–C(3″/5″)); 1.52–1.51 (*m*, H–C(4″)); 1.21–1.20 (*m*, H–C(5″)); 1.17 (*s*, H–C(9″/10″)). ^{13}C-NMR (125 MHz, CD_3OD): 168.7 (C(7″)); 155.6 (C(1)); 154.8 (C(3/5)); 141.5 (C(2″)); 135.0 (C(4)); 131.1 (C(1″)); 102.7 (C(1′)); 96.5 (C(2/6)); 77.7 (C(3′)); 75.5 (C(5′)); 74.8 (C(2′)); 72.8(C(8″)); 71.8 (C(4′)); 64.8 (6′)); 56.7 (*Me*O–C(3), *Me*O–C(5)); 61.3 (*Me*O–C(4)); 45.6 (C(4″)); 28.5 (C(3″)); 27.1(C(9″)); 26.4 (C(10″)); 26.3 (C(6″)); 24.4 (C(5″)). FAB-MS (neg.): 511 ($[M-H]^-$). HR-ESI-MS: 547.1954 ($[M+Cl]^-$, $C_{25}H_{36}ClO_{11}^-$; calc. 547.1946).

*(3*S*)-5-Guaiacyl-3-hydroxypentanoic Acid* (= *(3*S*)-3-Hydroxy-5-(4-hydroxy-3-methoxyphenyl)pentanoic Acid*; **2**). Pale amorphous powder. $[\alpha]_D^{25} = -6.8$ ($c = 1.8$, MeOH). UV (MeOH): 225 (3.66), 281(3.35). IR: 3422, 2935, 2856, 1710, 1602, 1517, 1399, 1273, 1033. ^{1}H-NMR (400 MHz, CD_3OD): 6.78 (*d*, *J* = 1.6, H–C(2′)); 6.69 (*d*, *J* = 8.0, H–C(5′)); 6.63 (*dd*, *J* = 1.6, 8.0, H–C(6′)); 3.90–3.86 (*m*, H–C(3)); 3.82 (*s*, MeO–C(3′)); 2.70–2.65 (*m*, H–C(5)); 2.60–2.55 (*m*, H–C(5)); 2.42 (*dd*, *J* = 2.4, 11.6, H–C(2)); 2.36 (*dd*, *J* = 6.4, 11.6, H–C(2)); 1.74–1.72 (*m*, CH_2(4)). ^{13}C-NMR (100 MHz, CD_3OD): 179.7 (C(1)); 148.8 (C(3′)); 145.4 (C(4′)); 135.2 (C(1′)); 121.8 (C(6′)); 116.1 (C(5′)); 113.2 (C(2′)); 69.4 (C(3)); 56.3 (MeO); 44.7 (C(2)); 42.7 (C(4)); 32.5 (C(5)). FAB-MS (neg.): 239 ($[M-H]^-$). HR-ESI-MS: 239.0915 ($[M-H]^-$, $C_{12}H_{15}O_5^-$; calc. 239.0919).

*8-β-*C*-Glucopyranosyl-5,7-dihydroxy-2-isobutylchromone* (= *(1*S*)-1,5-Anhydro-1-[5,7-dihydroxy-2-(2-methylpropyl)-4-oxo-4*H*-1-benzopyran-8-yl]-*D*-glucitol*; **3**). Pale yellow amorphous powder. $[\alpha]_D^{15} = -1.3$ ($c = 0.3$, MeOH). UV (MeOH): 208 (4.37), 251 (4.28), 297 (3.83). IR: 3405, 2959, 2872, 1660, 1619, 1427, 1274, 1086, 1023. ^{1}H-NMR (500 MHz, CD_3OD): 6.23 (*s*, H–C(6)); 6.06 (*s*, H–C(3)); 4.60 (*d*, *J* = 9.8, H–C(1′)); 4.09 (*t*, *J* = 9.0, H–C(2′)); 3.88 (*d*, *J* = 11.5, H–C(6′)); 3.66–3.64 (*m*, H–C(6′)); 3.48–3.45 (*m*, H–C(3′)); 3.42–3.40 (*m*, H–C(4′/5′)); 2.58 (*dd*, *J* = 6.5, 13.9, H–C(11)); 2.50 (*dd*, *J* = 6.5, 13.9, H–C(11)); 2.23–2.21 (*m*, H–C(12)); 1.03 (*d*, *J* = 6.6, Me(13)); 1.00 (*d*, *J* = 6.6, Me(14)). ^{13}C-NMR (125 MHz, CD_3OD): 184.2 (C(4)); 171.5 (C(2)); 165.1 (C(7)); 162.7 (C(5)); 158.4 (C(9)); 109.2 (C(3)); 105.6 (C(8)); 105.1 (C(10)); 100.2 (C(6)); 82.6 (C(5′)); 80.1 (C(3′)); 75.1 (C(1′)); 72.9 (C(2′)); 69.8 (C(4′)); 62.2 (C(6′)); 44.1 (C(11)); 28.2 (C(12)); 22.8 (C(13)); 22.5 (C(14)). FAB-MS (neg.): 395 ($[M-H]^-$). HR-ESI-MS: 395.1345 ($[M-H]^-$, $C_{19}H_{23}O_9^-$; calc. 395.1342).

Methanolysis of **1**. A soln. of **1** (2 mg) in 0.02M MeONa in MeOH (1 ml) was kept standing at r.t. for 12 h. The soln. was then subjected to CC over *MCI-gel CHP20P* (1.5 × 14 cm); H_2O, 60% and 100% MeOH/H_2O to give (−)-methyl 4-(1-hydroxy-1-methylethyl)cyclohex-1-ene-carboxylate (**1a**; (0.5 mg); colorless oil. $[\alpha]_D = -32.9$ ($c = 0.063$, $CHCl_3$), identified by co-TLC with authentic sample.

Synthesis of Methyl 3-Hydroxy-5-(4-hydroxy-3-methoxyphenyl)pentanonate (**2a**). According to the methodology described in [30], 5 ml of MeOH were added to AcCl (0.5 ml) dropwise, under stirring in an ice bath, to which, compound **2** (6 mg) and molecular sieve desiccant were added. The mixture was refluxed for 3.5 h (65°), and then passed through *MCI-gel CHP20P*; eluting with H_2O, 40% and 100%

MeOH/H_2O, to give **2a** (6 mg). Colorless oil. $[\alpha]_D = -22.1$ ($c = 0.1$, $CHCl_3$). FAB-MS (neg.): 253 ([$M-H$]$^-$).

*Preparation of (*R*)- and (S)-MTPA Esters of* **2a**. A soln. of **2a** (3 mg), (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA) or (*S*)-MTPA (6 mg), *N*,*N*-dicyclohexylcarbodiimide (DCC; 4 mg), and 4-(dimethylamino)pyridine (DMPA; 3 mg) in CH_2Cl_2 was stirred for 12 h at r.t. [31]. The mixture was purified by CC (SiO_2; $CHCl_3$/MeOH 98:2) to give (*R*)- or (*S*)-MTPA esters of **2a** (2 mg). The purified derivatives were dried and analyzed by means of ^{1}H-NMR and ^{1}H,^{1}H-COSY spectroscopies.

Cytotoxicity Assay. Five human cancer cell lines, breast cancer MCH-7, hepatocellular carcinoma SMMC-7721, human myeloid heukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells cultured in *RPMI-1640* or DMEM medium (*Dulbecco*'s Modified Eagle Medium; *Hyclone*, USA), supplemented with 10% fetal bovine serum (*Hyclone*, USA) in 5% CO_2 at 37°. The cytotoxicity assay was performed according to the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method in 96-well microplates. Briefly, 100 µl of adherent cells was seeded into each well of 96-well cell-culture plates and allowed to adhere for 12 h before drug addition, while suspended cells was seeded just before addition with an initial desity of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 µM in triplicates for 48 h, with cisplatin (*Sigma*, USA) as positive controls. After compound treatment, cell viability was detected, and a cell-growth curve was plotted. IC_{50} Values were calculated by *Reed* and *Muench*'s method.

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