Benzaldehyde Derivatives from *Eurotium rubrum*, an Endophytic Fungus Derived from the Mangrove Plant *Hibiscus tiliaceus*

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Four new (1—4) and seven known (5—11) benzaldehyde derivatives were characterized from the liquid fermentation cultures of *Eurotium rubrum*, an endophytic fungus that was isolated from the inner tissue of stems of the mangrove plant *Hibiscus tiliaceus*. The structures of these compounds were determined by extensive analysis of their spectroscopic data. Among these metabolites, compound 1, which was named as eurotirumin, possesses a new carbon skeleton with a cyclopentabenzopyran ring system.

Key words *Eurotium rubrum*; endophytic fungus; mangrove plant; *Hibiscus tiliaceus*; benzaldehyde derivative

Marine derived microorganisms have attracted significant attention for their potential of producing novel metabolites.[1)] Endophytic fungi are a large group of microorganisms which were defined as fungi colonizing healthy plant tissue without causing overt symptoms in or apparent injury to the host. Endophytes have been proven to be a well-established source for structurally diverse and biologically active secondary metabolites.[2—4)] Marine mangrove plants were proven to be a rich source of endophytic fungi. Many secondary metabolites with novel structures and biological activities have been characterized from mangrove-derived endophytic fungi.[5—8)]

In the course of our ongoing project directed toward the discovery of new natural products from endophytic fungi that were isolated from marine organisms from the Chinese sea coasts,[9—14)] we have investigated the chemical constituents of an endophytic fungal strain *Eurotium rubrum* that was isolated from the inner tissue of stems of the mangrove plant *Hibiscus tiliaceus* collected from Hainan island. This paper describes the isolation, structure elucidation, and cytotoxicity of four new (**1**—**4**) and seven known (**5**—**11**) benzaldehyde derivatives. To our knowledge, compound **1**, which was named as eurotirumin, possesses a new carbon skeleton with a cyclopentabenzopyran ring system.

Results and Discussion

The fungus *E. rubrum* was grown in potato-dextrose broth (PDB) media. The combined extracts from the culture broth and from the mycelium were fractionated by repeated column chromatography on silica gel, reversed-phase silica gel C_{18}, and Sephadex LH-20, as well as by preparative TLC, to afford eleven metabolites (**1**—**11**).

Compound **1** was obtained as a yellowish amorphous powder. The IR spectrum exhibited absorptions at 3442 (OH), 1729 (carbonyl), and 1632 cm^{-1} (double bond). The EI-MS of **1** displayed a molecular ion peak at *m/z* 316 $[M]^+$. The molecular formula of **1** was determined to be $C_{19}H_{24}O_4$ (8 degrees of unsaturation) on the basis of positive HR-ESI-MS (*m/z* 339.1572 $[M+Na]^+$, Calcd for $C_{19}H_{24}O_4Na$, 339.1572), which was in agreement with the ^{1}H- and ^{13}C-NMR spectral data of **1** (Tables 1, 2). The ^{1}H-NMR spectrum of **1** recorded in $CDCl_3$ (Table 1) revealed the presence of a phenolic hydroxyl proton at δ 12.04 (1H, s, OH-6) and an aldehyde proton at δ 10.32 (1H, s, H-7) in the lower field. Furthermore, an aromatic singlet at δ 6.97 (1H, s, H-4) and a further olefinic triplet at δ 5.29 (t, 1H, J=7.5 Hz, H-2″) were present in the aromatic and olefinic regions, respectively. In addition, the presence of two oxygenated methine proton signals at δ 4.72 (1H, m, H-2′) and 4.09 (1H, dq, J=10.1, 6.2 Hz, H-6′), two non-oxygenated methine proton signals at δ 2.78 (1H, dd, J=12.4, 4.4 Hz, H-1′) and 2.17 (1H, m, H-5′), three methylene proton signals at δ 3.29 (2H, d, J=7.5 Hz, H-1″), 1.87 (1H, m, H-3′a) and 2.40 (1H, m, H-3′b), and 1.26 (1H, m, H-4′a) and 1.94 (1H, m, H-4′b), three methyl signals with two singlets and one doublet at δ 1.75 (3H, s, H-4″), 1.67 (3H, s, H-5″), and 1.41 (3H, d, J=6.2 Hz, H-7′), were also present in the ^{1}H-NMR spectrum of **1** (Table 1). The ^{13}C-NMR spectrum of **1** exhibited 19 carbon signals attributable to three methyls, three methylenes, seven methines, and six quaternary carbon atoms according to the DEPT experiments (Table 2). Detailed comparison of 1D and 2D NMR spectral data of **1** with those of our recently reported data for chaetopyranin (**5**), a benzaldehyde derivative that was identi-

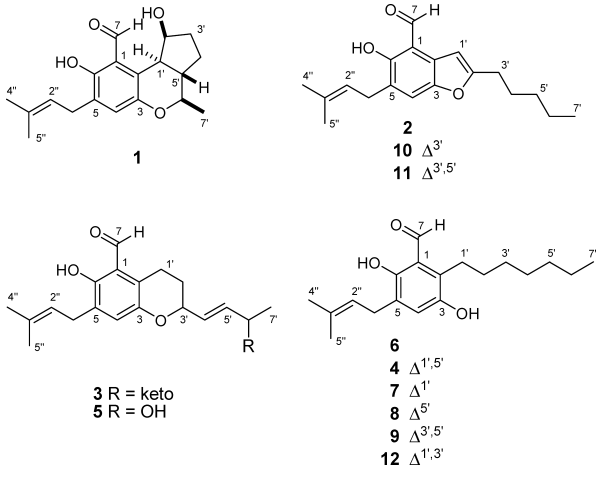

Fig. 1. Chemical Structures of Compounds **1**—**12**

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Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**4** (500 MHz)

Position	**1**[a)]	**1**[b)]	**2**[a)]	**3**[a)]	**4**[a)]
4	6.97 (s)	6.89 (s)	7.44 (s)	6.97 (s)	7.02 (s)
7	10.32 (s)	10.38 (s)	10.24 (s)	10.20 (s)	10.09 (s)
1′	2.78 (dd, 12.4, 4.4)	2.84 (dd, 12.5, 4.6)	6.65 (s)	3.11 (m)	6.44 (d, 16.2)
2′	4.72 (m)	4.78 (m)		2.21 (m), 1.88 (m)	5.90 (dt, 16.2, 6.9)
3′	1.87 (m) 2.40 (m)	1.84 (m) 2.40 (m)	2.77 (t, 7.6)	4.67 (ddd, 9.4, 4.3, 2.1)	2.40 (q, 6.8)
4′	1.26 (m) 1.94 (m)	1.28 (m) 1.90 (m)	1.75 (m)	6.81 (dd, 16.0, 4.4)	2.22 (q, 6.8)
5′	2.17 (m)	2.14 (m)	1.38 (m)	6.39 (dd, 16.0, 1.6)	5.43 (dt, 14.1, 6.2)
6′	4.09 (dq, 10.1, 6.2)	4.10 (dq, 10.1, 6.2)	1.38 (m)		5.50 (m)
7′	1.41 (d, 6.2)	1.35 (d, 6.2)	0.91 (t, 6.9)	2.30 (s)	1.69 (d, 7.5)
1″	3.29 (d, 7.5)	3.27 (d, 7.2)	3.40 (d, 7.4)	3.28 (d, 7.4)	3.31 (d, 7.4)
2″	5.29 (t, 7.5)	5.31 (t, 7.5)	5.34 (t, 7.4)	5.28 (t, 7.4)	5.29 (t, 7.4)
4″	1.75 (s)	1.73 (s)	1.78 (s)	1.75 (s)	1.75 (s)
5″	1.67 (s)	1.70 (s)	1.72 (s)	1.69 (s)	1.70 (s)
6-OH	12.04 (s)	12.15 (s)	11.66 (s)	11.98 (s)	11.71(s)
2′-OH		3.89 (d, 5.0)			

a) Measured in $CDCl_3$. *b*) Measured in acetone-d_6.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**4** (125 MHz)

Position	**1**[a)]	**1**[b)]	**2**[a)]	**3**[a)]	**4**[a)]
1	117.2 (s)	118.8 (s)	110.9 (s)	116.4 (s)	117.2 (s)
2	119.6 (s)	123.3 (s)	128.6 (s)	118.9 (s)	123.8 (s)
3	148.8 (s)	149.6 (s)	148.5 (s)	145.8 (s)	144.9 (s)
4	127.1 (d)	126.9 (d)	119.5 (d)	127.1 (d)	125.1 (d)
5	130.9 (s)	130.2 (s)	125.4 (s)	130.5 (s)	130.5 (s)
6	155.9 (s)	155.8 (s)	157.5 (s)	156.5 (s)	155.1 (s)
7	195.5 (d)	198.5 (d)	193.0 (d)	194.1 (d)	196.2 (d)
1′	48.1 (d)	48.6 (d)	98.5 (d)	20.0 (t)	120.9 (d)
2′	74.0 (d)	74.0 (d)	162.7 (s)	26.7 (t)	141.9 (d)
3′	35.2 (t)	36.4 (t)	28.6 (t)	73.4 (d)	33.1 (t)
4′	24.2 (t)	24.9 (t)	27.4 (t)	144.0 (d)	32.0 (t)
5′	44.7 (d)	45.8 (d)	31.3 (t)	130.3 (d)	130.2 (d)
6′	76.7 (d)	77.7 (d)	22.4 (t)	197.9 (s)	126.7 (d)
7′	20.3 (q)	20.7 (q)	13.9 (q)	27.5 (q)	17.8 (q)
1″	27.2 (t)	27.8 (t)	27.5 (t)	27.0 (t)	27.2 (t)
2″	121.1 (d)	122.7 (d)	121.5 (d)	120.9 (d)	121.1 (d)
3″	133.7 (s)	133.6 (s)	133.8 (s)	133.9 (s)	133.8 (s)
4″	25.8 (q)	25.9 (q)	25.7 (q)	25.7 (q)	25.7 (q)
5″	17.7 (q)	17.8 (q)	17.8 (q)	17.7 (q)	17.8 (q)

a) Measured in $CDCl_3$. *b*) Measured in acetone-d_6.

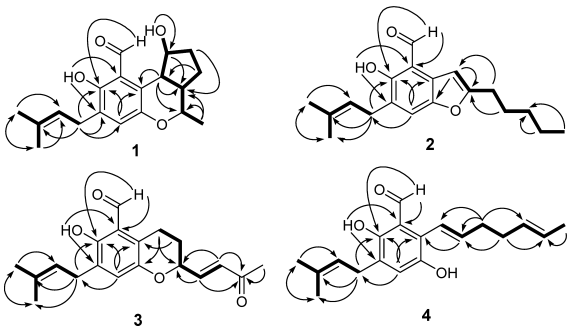

Fig. 2. Key HMBC (Arrow) and ^{1}H–^{1}H COSY (Bold Line) Correlations of Compounds **1**—**4**

fied from a marine algal-derived endophytic fungus *Chaetomium globosum*,[9] revealed that **1** was also a benzaldehyde derivative with a penta-substituted benzene ring system bearing a 3-methyl-2-butenyl at C-5 and a phenolic hydroxyl group at C-6.[9] In the ^{1}H–^{1}H COSY spectrum of **1**, the correlations from H-2′ to H-1′ and H-3′, from H-4′ to H-3′ and H-5′, from H-5′ to H-6′, and from H-6′ to H-7′, revealed contiguous sequence of the proton signals from H-1′ to H-7′ (Fig. 2). A further COSY correlation from H-1′ to H-5′ indicated that C-1′ and C-5′ were connected to form a five-membered carbon ring system, which was further confirmed by the observed HMBC correlations from H-1′ to C-5′ and C-6′, from H-2′ to C-5′, and from H-4′ to C-1′ (Fig. 2). Since the proton signal of OH-2′ was not observed in the ^{1}H-NMR spectrum that was recorded in $CDCl_3$, we re-measured the 1D- and 2D-NMR spectra of **1** by using of acetone-d_6 (Tables 1, 2). The hydroxyl proton signal at δ 3.89 (OH-2′) showed a COSY correlation with proton signal at δ 4.78 (H-2′). Furthermore, a ^2J C–H correlation from this proton to the carbon signal at δ 74.0 (C-2′) was also observed in the HMBC spectrum. These data indicated that a hydroxyl group was attached to C-2′. The presence of a benzopyran ring system in **1** was deduced by the number of oxygen atoms and oxygenated carbons as indicated by molecular formula and NMR data, respectively, as well as by the number of unsaturation.

The relative configuration of **1** was determined by the analysis of proton coupling constants. The coupling constant 12.4 Hz for H-1′ and H-5′ suggested the axial-orientation for H-1′ and H-5′, while the coupling constant 4.4 Hz for H-1′ and H-2′ suggested *cis*-configuration for both protons. The large coupling constant (10.1 Hz) for H-6′ and H-5′ indicated H-6′ to be axial. This is in agreement with the literature reports that in benzopyrans an equatorial orientation is preferred for the C-2 (in the case for **1**, C-6′) substitution.[9,15,16] However, the absolute configuration of **1** remains unknown.

From the above deductions, the structure of **1** was assigned to be 1,8-dihydroxy-4-methyl-7-(3-methyl-2-butenyl)-1,2,3,3a,4,9b-hexahydrocyclopenta[*c*]chromene-9-carbaldehyde, which was named as eurotirumin.

Compounds **2**—**4** were also obtained as yellowish amorphous powders. Detailed analyses of their NMR (Tables 1, 2) and MS data as well as by comparison with reported literature data revealed that all of these three compounds belonging to benzaldehyde derivatives and, similar to **1**, each of them possesses a penta-substituted benzene ring system bearing a 3-methyl-2-butenyl at C-5 and a phenolic hydroxyl

group at C-6.

The EI-MS of **2** exhibited a molecular ion peak at *m/z* 300 $[M]^+$. Its molecular formula was determined as $C_{19}H_{24}O_3$ on the basis of positive HR-ESI-MS (*m/z* 323.1612 $[M+Na]^+$, Calcd for $C_{19}H_{24}O_3Na$, 323.1623) which was in agreement with the ^{1}H- and ^{13}C-NMR spectral data of **2** (Tables 1, 2). Detailed comparison of the ^{1}H- and ^{13}C-NMR spectral data of **2** with those of 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3″-methyl-2″-butenyl)benzaldehyde (**10**) revealed that the structures of these two compounds are very similar,[9] except for two olefinic carbon signals of C-3′ (δ 118.4, d) and C-4′ (δ 135.4, d) in the ^{13}C-NMR of **10** were replaced by two methylene signals at δ 28.6 (t, C-3′) and 27.4 (t, C-4′), respectively, in **2**. This observation was strongly supported by the fact that the two olefinic proton signals appearing at δ 6.31 (dd, J=17.2, 1.4 Hz) for H-3′ and 6.51 (m) for H-4′ in **10** were absent in the ^{1}H-NMR spectrum of **2**. Instead, two two-proton signals with one triplet at δ 2.77 (J=7.6 Hz) for H-3′ and one multiplet at δ 1.75 for H-4′ were observed. The correlations from H-1′ to C-2′ and C-3′ as well as from H-3′ to C-1′ and C-2′ in the HMBC spectrum of **2** (Fig. 2) supported this deduction. Based on the above evidences, the structure of **2** was assigned to be 2-(2′,3-epoxy-1′-heptenyl)-6-hydroxy-5-(3″-methyl-2″-butenyl)benzaldehyde.

The EI-MS of **3** exhibited a molecular ion peak at *m/z* 314 $[M]^+$. Its molecular formula was determined as $C_{19}H_{22}O_4$ on the basis of positive HR-ESI-MS (*m/z* 315.1597 $[M+H]^+$, Calcd for $C_{19}H_{23}O_4$, 315.1596) which was in agreement with the ^{1}H- and ^{13}C-NMR spectral data of **3** (Tables 1, 2). Detailed comparison of the ^{1}H- and ^{13}C-NMR spectral data of **3** with those of **5** revealed that the structures of these two compounds are very similar.[9] However, the signals at δ 4.29 (m, H-6′) and δ 67.7 (d, C-6′) in **5** were replaced by a carbonyl signal at δ 197.9 (s, C-6′) in **3**. In the HMBC spectrum of **3**, the cross peaks from H-4′, H-5′, and H-7′ to the carbonyl carbon C-6′ confirmed the presence of a carbonyl group at C-6′ (Fig. 2). The relative configuration of **3** was determined by the analysis of proton coupling constants. The coupling constant 16.0 Hz indicated the *E*-geometry for the double bond at C-4′, while the coupling constant 9.4 Hz for H-3′ indicated a vicinal axial–axial coupling with H_{ax}-2′, which suggested the substitution at C-3′ to be equatorial. This is also in agreement with the literature reports that in benzopyrans an equatorial orientation is preferred for the C-2 (in the case for **3**, C-3′) substitution.[9,15,16] From the above deductions, the structure of **3** was assigned to be (*E*)-6-hydroxy-7-(3-methyl-2-butenyl)-2-(3-oxobut-1-enyl)chroman-5-carbaldehyde.

The molecular formula of **4** was determined as $C_{19}H_{24}O_3$ on the basis of negative HR-ESI-MS (*m/z* 299.1660 $[M-H]^-$, Calcd for $C_{19}H_{23}O_3$, 299.1647) which was in agreement with the ^{1}H- and ^{13}C-NMR spectral data of **4** (Tables 1, 2). Detailed comparison of the ^{1}H- and ^{13}C-NMR spectral data revealed that the chemical structure of **4** was very similar to dihydroauroglaucin (**12**) and isodihydroauroglaucin (**9**).[17,18] The only difference was observed with regard to the positions for the two double bonds in the heptadienyl side chain. In the ^{1}H–^{1}H COSY spectrum (Fig. 2) of **4**, one of the olefinic protons H-1′ (δ 6.44) correlated to H-2′ (δ 5.90). The latter was further connected to a spin system containing two methylenes H-3′ (δ 2.40) and H-4′ (δ 2.22), one double bond H-5′ (δ 5.43) and H-6′ (δ 5.50), and, finally, a methyl doublet for H-7′ (δ 1.69). These COSY correlations unambiguously indicated the presence of a hepta-1,5-dienyl group in **4**. The coupling constants 16.2 Hz and 14.1 Hz indicated the *E*-geometry for the double bonds at C-1′ and C-5′, respectively. From the above deductions, the structure of **4** was assigned to be 2-(1′,5′-heptadienyl)-3,6-dihydroxy-5-(3″-methyl-2″-butenyl)benzaldehyde.

In addition to the new compounds **1**—**4**, seven known benzaldehyde derivatives (**5**—**11**) were also isolated and identified. By comparison of their NMR data with those reported in the literature, the structures of these compounds were identified as chaetopyranin (**5**),[9] flavoglaucin (**6**),[19] aspergin (**7**),[19] isotetrahydroauroglaucin (**8**),[18] isodihydroauroglaucin (**9**),[18] 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl)benzaldehyde (**10**),[9] and 2-(2′,3-epoxy-1′,3′,5′-heptatrienyl)-6-hydroxy-5-(3-methyl-2-butenyl)benzaldehyde (**11**).[19]

Compounds **1**—**11** were tested for cytotoxic effects on the P-388, K-562 and HL-60 cell lines using the MTT method and on A-549 cell line using the SRB method. None of these compounds showed cytotoxic activities against any of the four cell lines (IC_{50}>10 μg/ml).

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were performed on a Nicolet NEXUE 470 infrared spectrophotometer. UV spectra were measured on a PuXi TU-1810 UV–visible spectrophotometer. 1D and 2D NMR were recorded on a Bruker Avance spectrometer with TMS as internal standard and chemical shifts were recorded as δ values (500 MHz for ^{1}H and 125 MHz for ^{13}C). Mass spectra were performed on a VG Autospec 3000 mass spectrometer. Silica gel (200—300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), reversed-phase silica gel C_{18} (40—75 μm, Fuji Silysia Chemical Ltd.) and Sephadex LH-20 (18—110 μm, Merck, Darmstadt, Germany) were used for open CC.

Fungal Material The endophytic fungus *Eurotium rubrum* was isolated from the inner tissue of stems of the mangrove plant *Hibiscus tiliaceus* that was collected from Hainan Island, China, in August, 2004, by using of a standard procedure.[9] Fungal identification was carried out by using the method as our previous report.[9] The sequence data derived from the fungal strain have been submitted and deposited at GenBank with accession number EU001331. BLAST search result showed that the sequence was the most similar (99%) to the sequence of *Eurotium rubrum* (compared to gb AY373891.1). The strain (seed culture) is preserved at 4 °C on potato dextrose agar slants at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences with accession number QEN-0407-G2. For chemical investigations, the fungal strain was static cultivated in potato-dextrose (PD) liquid media containing 50% (v/v) sea water (glucose 10 g/l, mannitol 20 g/l, peptone 5 g/l, yeast extract 3 g/l, and monosodium glutamate 3 g/l, pH 6.0) for 30 d at room temperature.

Extraction and Isolation The fermented whole broth (30 l) was filtered through cheesecloth to separate into culture broth and mycelia. The former was extracted three times with EtOAc (10 l each time) to give an extract, while the latter was homogenized and extracted three times with MeOH (3 l each time) to give another extract. Since the TLC and HPLC profiles of the two extracts were nearly identical, they were combined before further separation. The combined extract (70 g) was subjected to a column chromatography (CC, 120×8.0 cm, i.d.) over silica gel eluted with different solvents of increasing polarity to yield 14 fractions (Frs. 1—14) on the basis of TLC analysis. Fr. 1 was subjected to CC (50×2 cm, i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent to afford three subfractions (Frs. 1-1—1-3). Fr. 1-1 and Fr. 1-3 were further purified by preparative TLC (plate: 20×20 cm) on silica gel with petroleum ether–EtOAc (50 : 1) as developing solvents to give compounds **2** (23.7 mg), **10** (4.7 mg), and **11** (2.0 mg), respectively. Fr. 2 was further fractionated by CC (60×2.5 cm, i.d.) on silica gel eluted with petroleum ether–EtOAc (from 60 : 1 to 20 : 1) to yield 5 subfractions (Frs. 2-1—2-5). Fr. 2-5 was subjected to CC (50×2.0 cm, i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent and further purified

by preparative TLC (plate: 20×20 cm, developing solvents: petroleum ether–EtOAc, 20 : 1) on silica gel to yield compound **4** (1.8 mg). Fr. 3 was subjected to CC (50×2 cm i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent to yield compounds **6** (16.4 mg) and **7** (21.7 mg). Fr. 4 was fractionated by CC (60×3.0 cm, i.d.) on silica gel eluted with petroleum ether–EtOAc (from 50 : 1 to 10 : 1) to yield 5 sub-fractions (Frs. 4-1—4-5). Fr. 4-3 was subjected to CC (50×2 cm, i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent and further purified by preparative TLC (plate: 20×20 cm, developing solvents: petroleum ether–EtOAc, 20 : 1) on silica gel to yield compound **8** (417.0 mg). Fr. 4-5 was subjected to CC (60×2.0 cm, i.d.) on Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1) as solvent system to give compound **1** (1.9 mg). Fr. 5 was fractionated by CC (60×3.0 cm, i.d.) on silica gel eluted with petroleum ether–EtOAc (from 30 : 1 to 10 : 1) to yield 4 sub-fractions (Frs. 5-1—5-4). Fr. 5-3 was subjected to CC (50×2.0 cm, i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent and further purified by preparative TLC (plate: 20×20 cm, developing solvents: petroleum ether–EtOAc, 20 : 1) on silica gel to yield compound **9** (91.9 mg). Fr. 9 was further fractionated by CC (60×3.0 cm, i.d.) on silica gel washed with petroleum ether–EtOAc (from 5 : 1 to 1 : 1) to yield 7 sub-fractions (Frs. 9-1—9-7). Fr. 9-6 was subjected to CC (60×2.0 cm i.d.) on Sephadex LH-20 using $CHCl_3$–MeOH (2 : 1) as solvent system and further purified by CC (50×2.0 cm i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent to yield compound **3** (29 mg). Fr. 10 was further fractionated by CC (60×3.5 cm i.d.) on silica gel eluted with petroleum ether–EtOAc (from 5 : 1 to 1 : 1) to yield 7 sub-fractions (Frs. 10-1—10-7). Fr. 10-6 was subjected to CC (50×2.0 cm i.d.) on reversed-phase silica gel C_{18} using MeOH as an eluent and further purified by preparative TLC (plate: 20×20 cm, developing solvents: petroleum ether–Me_2CO, 3 : 1) on silica gel to yield compound **5** (35.9 mg).

Compound **1**: Yellowish amorphous powder; $[\alpha]_D^{25}$ +8.8° (c=0.13, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ε): 393 (2.54), 277 (2.89), 227 (3.52); IR (KBr) ν_{max} cm^{-1}: 3442, 2930, 1729, 1632, 1449, 1291; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; EI-MS m/z 316 $[M]^+$ (10), 297 (100), 283 (39), 255 (22), 242 (23), 229 (16), 199 (14), 128 (8), 91 (7), 77 (7); HR-ESI-MS (positive) m/z 339.1572 $[M+Na]^+$ (Calcd for $C_{19}H_{24}O_4Na$, 339.1572).

Compound **2**: Yellowish amorphous powder; UV λ_{max} ($CHCl_3$) nm (log ε): 377 (3.79), 312 (3.99), 242 (4.15); IR (KBr) ν_{max} cm^{-1}: 2959, 2924, 2854, 1642, 1619, 1572, 1421, 1293, 947, 733; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; EI-MS m/z 300 $[M]^+$ (47), 285 (20), 257 (24), 245 (100), 243 (18), 159 (19); HR-ESI-MS (positive) m/z 323.1612 $[M+Na]^+$ (Calcd for $C_{19}H_{24}O_3Na$, 323.1623).

Compound **3**: Yellowish amorphous powder; $[\alpha]_D^{25}$ +1.5° (c=0.27, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ε): 386 (3.62), 275 (4.02), 224 (3.41); IR (KBr) ν_{max} cm^{-1}: 3394, 2963, 2932, 2850, 1673, 1638, 1429, 1297, 1266, 1126, 982, 757; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; EI-MS m/z 314 $[M]^+$ (46), 296 (10), 271 (15), 240 (21), 215 (27), 175 (39), 149 (100), 97 (49), 95 (62), 83(38), 71 (40); HR-ESI-MS (positive) m/z 315.1597 $[M+H]^+$ (Calcd for $C_{19}H_{23}O_4$, 315.1596).

Compound **4**: Yellowish amorphous powder; UV λ_{max} ($CHCl_3$) nm (log ε): 382 (3.33), 242 (3.84), 219 (3.39); IR (KBr) ν_{max} cm^{-1}: 3419, 2929, 1651, 1440, 1285, 974; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-ESI-MS (negative) m/z 299.1660 $[M-H]^-$ (Calcd for $C_{19}H_{23}O_3$, 299.1647).

Cytotoxicity Assays Cytotoxic assay toward the P-388 mouse leukemia cell lines, K-562 human leukemia cell lines, and HL-60 human promyelocytic leukemia cell lines was tested using the MTT method, while the SRB method was used for the A-549 human pulmonary epithelial cell line.[20)]

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