Ardisiphenols and Other Antioxidant Principles from the Fruits of *Ardisia colorata*

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Novel alkylphenols, ardisiphenols A—C (1—3) and a novel bergenin derivative, demethoxybergenin (10) were isolated from the fruits of *Ardisia colorata* (Myrsinaceae), together with known alkylresorcinols (4—6), embelin (7), myricetin (8), quercetin (9), bergenin (11), norbergenin (12), kaempferol (13), quercetin-3-*O*-β-D-glucopyranoside (14) and gallic acid (15). Their structures were determined by NMR, MS(/MS) analyses and other spectroscopic methods. Ardisiphenols showed moderate scavenging activities toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and showed cytotoxicity against the murine breast cancer cell line, FM3A.

Key words *Ardisia colorata*; Myrsinaceae; ardisiphenol; bergenin; antioxidant; cytotoxicity

In recent years, there has been increasing evidence that reactive oxygen species (ROS) are associated with pathological conditions such as atherosclerosis[1] and carcinogenesis,[2] as well as with aging.[3] Thus, a lot of attention has focused on dietary antioxidants which may have a potential for therapeutic use and prevention of these diseases.

Ardisia colorata ROXB. (Myrsinaceae) is a large shrub which has been used as a folk medicine to treat liver disease, cough and diarrhea in Thailand. An earlier phytochemical study on the bark of this plant reported the isolation of rapanone and ilexol.[4] In the course of screening for antioxidants in Thai medicinal plants, the extract of the fruits of *A. colorata* showed relatively high scavenging activity toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in TLC autographic assay.

In this paper, we describe the isolation and structural elucidation of newly isolated ardisiphenols A—C (**1**—**3**), demethoxybergenin (**10**) and 11 known compounds from the fruits of this plant. Their antioxidant activities and cytotoxicities against the murine breast cancer cell line, FM3A, were also investigated.

Results and Discussion

The 75% ethanol extract of the dried fruits of *A. colorata* was separated into *n*-hexane, EtOAc, *n*-BuOH and H_2O-soluble fractions. They were fractionated by repeated column chromatographies (CC) according to the activity in the TLC autographic assay.[5] *n*-Hexane-soluble fraction yielded ardisiphenols (**1**—**3**) and alkylresorcinols (**4**—**6**), as previously reported.[6] The EtOAc-soluble fraction afforded embelin (**7**)[7] as the major constituent, myricetin (**8**)[8] and quercetin (**9**).[9] A new bergenin derivative, demethoxybergenin (**10**) was isolated from the *n*-BuOH-soluble fraction, together with bergenin (**11**),[10,11] norbergenin (**12**),[10,11] kaempferol (**13**),[9] quercetin 3-*O*-β-D-glucopyranoside (**14**)[12] and gallic acid (**15**).[13]

Ardisiphenol A (**1**) was obtained as a colorless oil which darkened on exposure to air. The molecular formula of **1** was determined to be $C_{23}H_{38}O_4$ from high resolution (HR) FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals of two *meta* coupled aromatic protons at δ 6.13 (d, J=2.7 Hz, H-5) and 6.21 (d, J=2.7 Hz, H-3), and signals due to a long alkyl side chain at δ 0.89 (3H, t, J=7.0 Hz, H-15′), 1.28 (24H, overlapped, H-3′—14′), 1.50 (homobenzyl 2H, m) and 2.35 (benzyl 2H, t, J=7.3 Hz). The ^{13}C-NMR spectrum also indicated aromatic and alkyl components. Signals of an acetoxyl group were also observed. Electron impact (EI)-MS showed an intense peak at *m*/*z* 140 corresponding to benzylic fragmentation of a deacetylated ion of **1** and a base peak of a deacetylated ion of **1** at *m*/*z* 336 [M−Ac+H]$^+$. Therefore, **1** was assumed to be an alkylphenol bearing two *meta* coupled protons, two hydroxyl groups, an acetoxyl group and a linear C_{15} alkyl chain. The position of the hydroxyl groups was determined by comparison of its NMR data with those of **2**, which was determined as described below. The structure of **1** was finally concluded to be 6-*n*-pentadecyl-1,2,4-trihydroxybenzene-1-*O*-acetate (Fig. 1).

Ardisiphenol B (**2**) showed similar ^{1}H- and ^{13}C-NMR

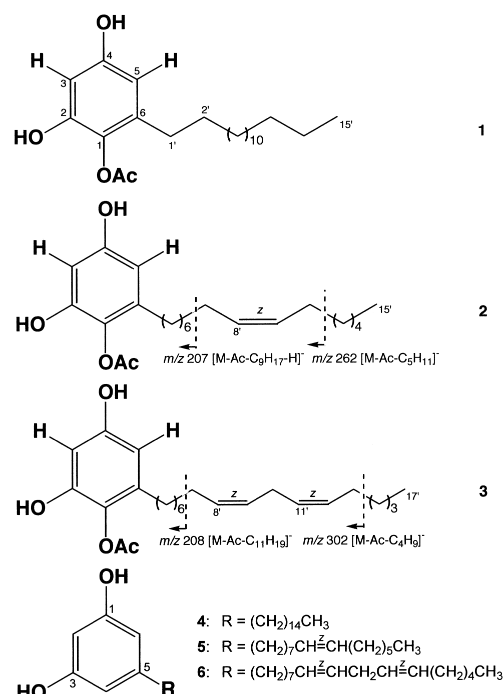

Fig. 1. Structures of **1**—**6** and Fragment Ions Observed in the Negative FAB-MS/MS Spectra of **2** and **3**

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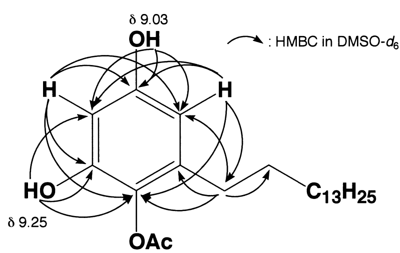

Fig. 2. Important HMBC Correlations for **2**

spectra to those of **1**, except for a set of olefin signals [δ 5.33 (2H, overlapped, H-8′, -9′), 130.81, 130.86 (C-8′, -9′)] and allylic methylene signals [δ 2.02 (4H, overlapped, H-7′, -10′), 28.13, 28.16 (C-7′, -10′)]. The molecular formula of **2**, $C_{23}H_{36}O_4$, which was two protons less than **1**, also indicated the existence of a C_{15} monounsaturated alkenyl chain. The position of the double bond was determined by negative FAB-MS/MS for the precursor ion at *m/z* 333 ($[M-Ac]^-$). Fragment ions were observed at *m/z* 262 ($[M-Ac-C_5H_{11}]^-$) and 207 ($[M-Ac-C_9H_{17}-H]^-$) resulting from the allylic cleavage of the 8′-alkenyl side chain (Fig. 1). The stereochemistry of the double bond was determined to be *Z* from the allylic (δ 28.13, 28.16) and olefinic (δ 130.81, 130.86) carbon signals, compared with the ^{13}C-NMR data of 3-(8′*Z*-heptadecenyl)-1,2-dimethoxybenzene (allylic carbons: δ 27.18, olefinic carbons: δ 129.82 and 129.87) and its 8′*E* isomer (allylic carbons: δ 32.58, olefinic carbons: δ 130.28 and 130.34).[14] Thus, it is proved that **2** has an 8′*Z*-pentadecenyl side chain.

The position of the phenolic hydroxyl groups for **1** and **2** was deduced from the analysis of the heteronuclear multiple bond connectivity (HMBC) spectrum of **2** which was measured in dimethylsulfoxide (DMSO)-d_6 (Fig. 2). Hydroxyl proton signals were observed at δ 9.03 (1H, s, C-4-OH) and 9.25 (1H, s, C-2-OH). The HMBC spectrum of **2** showed correlations between the hydroxyl proton (δ 9.03) and C-3, -4 and -5, and between the hydroxyl proton (δ 9.25) and C-1, -2 and -3. Thus, the hydroxyl groups were located at C-2 and C-4, respectively, and the acetoxyl group was proposed to be located at C-1. Finally, **2** was elucidated as 6-(8′*Z*-pentadecenyl)-1,2,4-trihydroxybenzene-1-*O*-acetate, namely, the 8′*Z*-enyl congener of **1**.

The ^{1}H- and ^{13}C-NMR spectra of ardisiphenol C (**3**) revealed the same phenolic structure as **1** and **2**. The molecular formula was determined to be $C_{25}H_{38}O_4$ from HR FAB-MS, indicating the existence of a diolefinic structure in the C_{17} side chain. Furthermore, bisallylic methylene signals at δ 2.75 (2H, t, J=6.7 Hz, H-10′) and 25.6 (C-10′) in the NMR spectra confirmed that two double bonds were separated by a methylene group. The negative FAB-MS/MS applied to the precursor ion at *m/z* 359 ($[M-Ac]^-$) showed ions at *m/z* 302 ($[M-Ac-C_4H_9]^-$) and 208 ($[M-Ac-C_{11}H_{19}]^-$), resulting from allylic cleavage of the 8′,11′-dienyl side chain (Fig. 1). The structure of the side chain of **3**, including the stereochemistry, is the same as that of 3-(8′*Z*,11′*Z*-heptadecadienyl)-1,2-dimethoxybenzene from consideration of the NMR data.[14] Thus, the above data lead us to propose the structure of **3** as 6-(8′*Z*,11′*Z*-heptadecadienyl)-1,2,4-tetrahydroxybenzene-1-*O*-acetate.

Compounds **4**, **5** and **6** were identified as 5-*n*-pentadecyl-1,3-benzenediol,[15] its 8′*Z*-enyl congener[15] and 5-(8′*Z*,11′*Z*-heptadecadienyl)-1,3-benzenediol,[16] respectively, by NMR and FAB-MS/MS analyses (Fig. 1). These compounds are known as alk(en)ylresorcinols, and are often found in plants of the families Ginkgoaceae, Anacardiaceae, Gramineae and Proteaceae. Recently, similar homologues were reported as constituents of *Ardisia* species (*A. silvestris*, *A. gigantifolia*).[17]

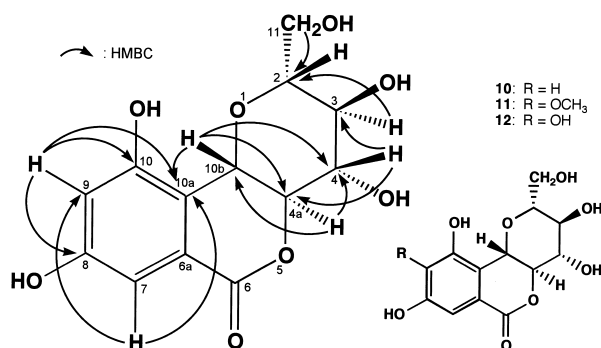

Fig. 3. Structures of **10**—**12** and HMBC Correlations for **10**

Compound **10** was isolated as colorless needles, along with the structurally related compounds **11** and **12**. The molecular formula of **10** was determined to be $C_{13}H_{14}O_8$ from HR FAB-MS. The ^{1}H-NMR data of **10** were similar to those of **11**, but *meta* coupled signals at δ 6.52 (H-9) and 7.08 (H-7) (each 1H, d, J=2.4 Hz) were observed in the spectrum of **10** instead of the singlet signal at δ 7.08 (1H, s, H-7) in the spectrum of **11**. Furthermore, the signals of the methoxyl group of **11** [(δ 3.89 (3H, s, C-9-OC$\underline{H}_3$), 60.9 (C-9-O$\underline{C}H_3$)] were not observed in the spectrum of **10**. In the HMBC experiment for **10** (Fig. 3), correlations were observed between the proton at δ 6.52 (H-9) and C-8, -10 and -10a, and between the proton at δ 7.08 (H-7) and C-9 and -10a. These data confirmed the positions of two *meta* coupled protons at the 7- and 9-positions. Thus, the planar structure of **10** was proposed to be demethoxybergenin. The NMR data for the asymmetric carbons of **10** were very close to those of **11**, and the specific rotation of **10** ($[\alpha]_D^{20}$: $-22.7°$, c=0.08, MeOH) was comparable to those of **11** ($[\alpha]_D^{20}$: $-21.4°$, c=0.29, MeOH) and **12** ($[\alpha]_D^{25}$: $-22.1°$, c=0.43, MeOH). These data indicate that they have the same configuration, as shown in Fig. 3. Thus, compound **10** was determined to be demethoxybergenin.

Bergenin is a *C*-glucoside and occurs in many plants,[18] such as *Bergenia crassiflora* (Saxifragaceae), *Mallotus japonicus* (Euphorbiaceae), and also *Ardisia hortorum* (Myrsinaceae). In bergenin biosynthesis, gallic acid (**15**) may be the glucosyl acceptor to be the precursor of bergenin from the result of the [^{14}C]-glucose incorporation experiment in *Saxifraga stolonifera* leaves.[19] Considering the structure of **10**, it may be that α-resorcylic acid is the glucosyl acceptor to be the precursor of **10**.

The DPPH radical scavenging activity was evaluated as IC_{50} and Trolox equivalent values[20] for the isolated compounds, as shown in Table 1. Ardisiphenols (**1**—**3**) showed moderate activity which was slightly stronger than that of alkylresorsinols (**4**—**6**), but the activity of ardisiphenols (**1**—**3**) was maximum at around 60 μM. The activity of alkylresorcinols (**4**—**6**) was not directly proportional to the concentra-

Table 1. DPPH Radical Scavenging Activities of Isolated Compounds from *A. colorata*

	IC_{50} value (μM)	Trolox equiv. (mM)
Ardisiphenol A (**1**)	47%[a]	0.5
Ardisiphenol B (**2**)	51%[a]	0.6
Ardisiphenol C (**3**)	51%[a]	0.6
Alkylresorcinol A (**4**)	90	n.d.
Alkylresorcinol B (**5**)	87	n.d.
Alkylresorcinol C (**6**)	80	n.d.
Embelin (**7**)	23.3±0.5	1.0
Myricetin (**8**)	16.0±0.4	1.6
Quercetin (**9**)	11.6±0.7	2.0
Demethoxybergenin (**10**)	—	—
Bergenin (**11**)	131	0.3
Norbergenin (**12**)	13.4±1.1	2.0
Kaempferol (**13**)	35.7±0.3	0.9
Quercetin 3-*O*-β-D-glucopyranoside (**14**)	17.3±0.6	1.4
Gallic acid (**15**)	12.4±0.2	2.0
Trolox	25.4±0.8	1.0

a) % inhibition at 60 μM. n.d.: not determined.

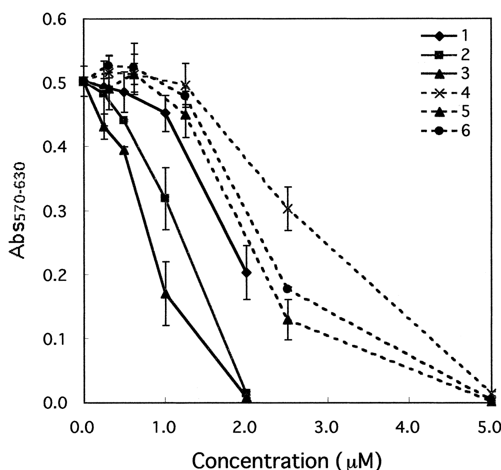

Fig. 4. Cytotoxicities of Ardisiphenols A—C (**1**—**3**) and Alkylresorcinols A—C (**4**—**6**) for the Murine Breast Cancer Cell Line, FM3A

Each value represents the mean±S.D. of triplicates.

tion, and that made it impossible to calculate the Trolox equivalent values. Quercetin (**9**), norbergenin (**12**) and gallic acid (**15**) showed strong activity. Embelin (**7**), the major constituent of this plant, showed activity comparable to Trolox. Among the bergenin related compounds (**10**—**12**), demethylation of **11** greatly enhanced the activity, and **10** showed no activity, probably due to the loss of a catechol function. Thus, it is determined that there are a variety of antioxidants in the fruits of this plant. Furthermore, it has been reported that bergenin derivatives have hepatoprotective activity against CCl_4 and galactosamine cytotoxicity in primary cultured rat hepatocytes.[21] These results may partly account for the folkloric use of this plant.

Because alkylresorcinols are known to have cytotoxicity,[22] this was determined for the alkylphenols (**1**—**6**) and bergenin derivatives (**10**—**12**) against the murine breast cancer cell line, FM3A (Fig. 4). Ardisiphenols A—C (**1**—**3**) showed potent cytotoxicity (IC_{50}: 1.8, 1.2, 0.5 μM, respectively), while **4**—**6** had weaker activity (IC_{50}: 2.8, 2.0, 2.2 μM, respectively). Compounds (**2**, **3**, **5**, **6**) which had either an alkenyl or alkadienyl side chain, showed stronger activity than two alkylphenols (**1**, **4**). The presence of an acetoxyl group in ardisiphenols and the unsaturated long chain might be important for the cytotoxicity. Among the isolated bergenin derivatives, **11** showed weak activity (IC_{50}: 44 μM), while the cytotoxicities of **10** and **12** were not obviously observed until 100 μM.

Experimental

General Melting points were determined on a Yanako melting point apparatus and are uncorrected. UV spectra were recorded with a Hitachi U-3200 spectrophotometer. IR spectra were recorded with a JASCO FT/IR-230 spectrophotometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a JEOL JNM-A400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) or a JEOL JNM-A500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) with tetramethylsilane (TMS) as an internal standard. EI-MS were taken on a JEOL JMS-GC-MATE mass spectrometer. FAB-MS(/MS) were taken on a JEOL JMS-HX110A mass spectrometer in an *m*-nitrobenzylalcohol (NBA) matrix in the positive or negative mode. Column chromatography was carried out on Kieselgel 60 (70—230 mesh, 230—400 mesh) (Merck) and Cosmosil C_{18}-OPN (Nacalai Tesque). Analytical HPLC was carried out on the TSK gel ODS120A (4.6 i.d.×150 mm) (TOSOH). For preparative HPLC, an Inertsil ODS column (20 i.d.×250 mm) (gaskurokogyo) was used (solvent system: MeOH/0.2%AcOH 85/15). TLC was performed on precoated silica gel 60 F_{254} (0.25 mm) (Merck) or RP-18 F_{254} (0.25 mm) (Merck), and spots were detected by UV (254 nm) or by 50% H_2SO_4 spraying reagent followed by heating.

Plant Material The fruits of *Ardisia colorata* (Myrsinaceae) were collected at Rayong Province, Thailand in May 1998. A voucher specimen is deposited in the herbarium, Graduate School of Pharm. Sci., Chiba University, Japan.

Extraction and Isolation The dried fruits of *A. colorata* (500 g) were extracted with 75% EtOH at room temperature. The solvent was evaporated under reduced pressure to give the extract (62 g). The extract was partitioned with H_2O–*n*-hexane, and the remaining H_2O layer was successively extracted with EtOAc and *n*-BuOH. A portion (5.2 g) of the *n*-hexane-soluble fraction (12 g) was subjected to silica gel column chromatography (2.5 i.d.×37 cm) with $CHCl_3$–MeOH of increasing polarity to give 8 fractions. Fr. 4 was applied to silica gel column chromatography (1.8 i.d.×23 cm) with *n*-hexane/EtOAc of increasing polarity to give 7 fractions. Subfr. 4 was subjected to ODS HPLC (MeOH/0.2%AcOH 85/15) to provide ardisiphenol A (**1**, 0.043%/g dried fruits), B (**2**, 0.084%), and C (**3**, 0.058%). Subfr. 2 was also subjected to ODS HPLC (MeOH/0.2%AcOH 85/15) to provide alkylresorcinols (**4**, 0.022%; **5**, 0.053%; **6**, 0.037%). Subsequently, a portion (2.0 g) of the EtOAc-soluble fraction (5 g) was separated by silica gel column chromatography (2.2 i.d.×22 cm) with $CHCl_3$/MeOH of increasing polarity to give 7 fractions. Fraction 1 was recrystallized to give embelin (**7**, 0.32%) and fr. 4 was subjected to ODS column chromatography (1.2 i.d.×16 cm) with acetone/H_2O to afford myricetin (**8**, 0.0016%) and quercetin (**9**, 0.0043%). A portion (4.5 g) of the *n*-BuOH-soluble fraction (7 g) was separated by silica gel column chromatography (2.6 i.d.×30 cm) with $CHCl_3$/MeOH/H_2O of increasing polarity to give 10 fractions. Fraction 3 was purified by ODS HPLC (MeOH/H_2O 55/45) to give kaempferol (**13**, 0.0013%), and fr. 6 was separated by ODS HPLC (MeOH/H_2O 20/80) to give bergenin (**11**, 0.0043%) and demethoxybergenin (**10**, 0.0036%). Fraction 7 was subjected to ODS column chromatography (2.0 i.d.×17 cm) with MeOH/H_2O to afford gallic acid (**15**, 0.0053%), norbergenin (**12**, 0.017%) and quercetin 3-*O*-β-D-glucopyranoside (**14**, 0.0020%).

Ardisiphenol A (**1**): Colorless oil which darkens on exposure to air, UV λ_{max} (MeOH) nm (log ε): 224 (3.85), 279 (3.44). IR (KBr) cm^{-1}: 3363, 2922, 2852, 1718, 1238, 1184. Positive HR FAB-MS *m/z*: 417.2390 $[M+K]^+$ (Calcd for $C_{23}H_{38}O_4K$: 417.2407). EI-MS *m/z* (rel. int.): 378 $[M]^+$ (11), 336 $[M-Ac+H]^+$ (100), 140 (83). ^{1}H-NMR (500 MHz, CD_3OD): δ: 0.89 (3H, t, J=7.0 z, H-15′), 1.28 (24H, overlapped, methylene H-3′—14′), 1.50 (2H, m, H-2′), 2.25 (3H, s, Ac), 2.35 (2H, t, J=7.3 Hz, H-1′), 6.13 (1H, d, J=2.7 Hz, H-5), 6.21 (1H, d, J=2.7 Hz, H-3). ^{13}C-NMR (125 MHz, CD_3OD): δ: 14.4 (C-15′), 20.5 (OCO$\underline{C}H_3$), 23.7 (C-14′), 30—31 (methylene C-1′—12′), 33.1 (C-13′), 102.4 (C-3), 107.9 (C-5), 131.6 (C-1), 137.4 (C-6), 150.6 (C-2), 156.5 (C-4), 171.6 (O$\underline{C}$OCH$_3$).

Ardisiphenol B (**2**): Colorless oil which darkens on exposure to air, UV λ_{max} (MeOH) nm (log ε): 224 (3.85), 279 (3.39). IR (KBr) cm^{-1}: 3393,

2925, 2854, 1735, 1248, 1184. Positive HR FAB-MS *m/z*: 415.2240 $(M+K)^+$ (Calcd for $C_{23}H_{36}O_4K$: 415.2250). Negative FAB-MS *m/z*: 375 $[M-H]^-$, 333 $[M-Ac]^-$. Negative FAB-MS/MS (applied to *m/z* 333 $[M-Ac]^-$ ion) *m/z* (rel. int.): 333 $[M-Ac]^-$ (100), 262 $[M-Ac-C_5H_{11}]^-$ (0.6), 207 $[M-Ac-C_9H_{17}-H]^-$ (0.3). ^{1}H-NMR (500 MHz, CD_3OD): δ: 0.89 (3H, t, J=7.0 Hz, H-15′), 1.31 (16H, overlapped, methylene), 1.50 (2H, m, H-2′), 2.02 (4H, overlapped, H-7′, -10′), 2.25 (3H, s, Ac), 2.35 (2H, t, J=7.3 Hz, H-1′), 5.33 (2H, overlapped, H-8′, -9′), 6.13 (1H, d, J=2.8 Hz, H-3), 6.21 (1H, d, J=2.8 Hz, H-5). ^{13}C-NMR (125 MHz, CD_3OD): δ: 14.4 (C-15′), 20.6 (OCO$\underline{C}H_3$), 23.7 (C-14′), 28.13, 28.16 (C-7′, -10′), 30—31 (methylene), 31.3 (C-1′), 32.9 (C-13′), 102.4 (C-3), 107.9 (C-5), 130.81, 130.86 (C-8′, -9′), 131.6 (C-1), 137.3 (C-6), 150.6 (C-2), 156.4 (C-4), 171.6 (O$\underline{C}$OCH$_3$). ^{1}H-NMR (500 MHz, DMSO-d_6): δ: 0.84 (3H, t, J=7.0 Hz, H-15′), 1.24 (16H, overlapped, methylene), 1.40 (2H, m, H-2′), 1.97 (4H, overlapped, H-7′, -10′), 2.18 (3H, s, Ac), 2.25 (2H, t, J=7.3 Hz, H-1′), 5.31 (2H, overlapped, H-8′, -9′), 6.03 (1H, d, J=2.7 Hz, H-3), 6.16 (1H, d, J=2.7 Hz, H-5), 9.03 (1H, s, C-4-OH), 9.25 (1H, s, C-2-OH). ^{13}C-NMR (125 MHz, DMSO-d_6): δ: 13.9 (C-15′), 20.3 (OCO$\underline{C}H_3$), 22.1 (C-14′), 26.6 (C-7′, -10′), 28—29 (methylene), 31.1 (C-13′), 101.2 (C-3), 106.1 (C-5), 129.61*, 129.63* (C-8′, -9′), 129.53* (C-1), 135.4 (C-6), 149.3 (C-2), 155.0 (C-4), 168.8 (O$\underline{C}$OCH$_3$). *: interchangeable.

Ardisiphenol C (**3**): Colorless oil which darkens on exposure to air, UV λ_{max} (MeOH) nm (log ε): 223 (3.89), 280 (3.45). IR (KBr) cm^{-1}: 3398, 2926, 2855, 1749, 1239, 1183. Positive HR FAB-MS *m/z*: 441.2380 $(M+K)^+$ (Calcd for $C_{25}H_{38}O_4K$: 441.2407). Negative FAB-MS *m/z*: 401 $[M-H]^-$, 359 $[M-Ac]^-$. Negative FAB-MS/MS (applied to *m/z* 359 $[M-Ac]^-$ ion) *m/z* (rel. int.): 359 $[M-Ac]^-$ (100), 302 $[M-Ac-C_4H_9]^-$ (6), 208 $[M-Ac-C_{11}H_{19}]^-$ (2). ^{1}H-NMR (500 MHz, $CDCl_3$): δ: 0.86 (3H, t, J=7.0 Hz, H-17′), 1.27 (14H, overlapped, methylene), 1.47 (2H, m, H-2′), 2.04 (4H, overlapped, H-7′, -13′), 2.30 (3H, s, Ac), 2.35 (2H, t, J=7.3 Hz, H-1′), 2.75 (2H, t, J=6.7 Hz, H-10′), 5.33 (4H, overlapped, H-8′, -9′, -11′, -12′), 6.18 (1H, br s, H-3), 6.18 (1H, br s, H-5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ: 14.1 (C-17′), 20.6 (OCO$\underline{C}H_3$), 22.6 (C-16′), 25.6 (C-10′), 27.18, 27.21 (C-7′, -13′), 29—30 (methylene), 30.3 (C-1′), 31.5 (C-15′), 102.3 (C-3), 108.4 (C-5), 127.9, 128.0, 130.1, 130.2 (C-8′, -9′, -11′, -12′), 130.8 (C-1), 136.6 (C-6), 147.8 (C-2), 153.7 (C-4), 170.8 (O$\underline{C}$OCH$_3$).

Compound **4**: Colorless oil which darkens on exposure to air, EI-MS *m/z* (rel. int.): 320 $[M]^+$ (6), 124 (100). ^{1}H-NMR (500 MHz, CD_3OD): δ: 0.89 (3H, t, J=7.1 Hz, H-15′), 1.28 (24H, overlapped, methylene), 1.55 (2H, m, H-2′), 2.42 (2H, t, J=7.6 Hz, H-1′), 6.07 (1H, t, J=2.2 Hz, H-2), 6.11 (2H, d, J=2.2 Hz, H-4, -6).

Compound **5**: Colorless oil which darkens on exposure to air, EI-MS *m/z* (rel. int.): 318 $[M]^+$ (95), 124 (99). Negative FAB-MS *m/z*: 317 $[M-H]^-$. FAB-MS/MS (applied to *m/z* 317 $[M-H]^-$ ion) *m/z* (rel. int.): 317 $[M-H]^-$ (100), 245 $[M-C_5H_{11}-2H]^-$ (0.4), 191 $[M-C_9H_{17}-2H]^-$ (0.4). ^{1}H-NMR (500 MHz, CD_3OD): δ: 0.89 (3H, t, J=7.1 Hz, H-15′), 1.31 (16H, overlapped, methylene), 1.55 (2H, m, H-2′), 2.01 (4H, overlapped, H-7′, -10′), 2.42 (2H, t, J=7.3 Hz, H-1′), 5.33 (2H, overlapped, H-8′, -9′), 6.07 (1H, t, J=2.2 Hz, H-2), 6.11 (2H, d, J=2.2 Hz, H-4, -6). ^{13}C-NMR (125 MHz, CD_3OD): δ: 14.4 (C-15′), 23.7 (C-14′), 30—32 (methylene), 28.11, 28.15 (C-7′, -10′), 32.9 (C-13′), 37.0 (C-1′), 100.9 (C-2), 107.9 (C-4, -6), 130.84, 130.82 (C-8′, -9′), 146.3 (C-5), 159.2 (C-1, -3).

Compound **6**: Colorless oil which darkens on exposure to air, EI-MS *m/z* (rel. int.): 344 $[M]^+$ (8), 124 (100). Negative FAB-MS *m/z*: 343 $[M-H]^-$. FAB-MS/MS (applied to *m/z* 343 $[M-H]^-$ ion) *m/z* (rel. int.): 343 $[M-H]^-$ (100), 285 $[M-C_4H_9-2H]^-$ (0.2), 191 $[M-C_{11}H_{19}-2H]^-$ (0.2). ^{1}H-NMR (500 MHz, CD_3OD): δ: 0.89 (3H, t, J=7.0 Hz, H-17′), 1.32 (14H, overlapped, methylene), 1.57 (2H, m, H-2′), 2.06 (4H, overlapped, H-7′, -13′), 2.42 (2H, t, J=7.0 Hz, H-1′), 2.76 (2H, t, J=6.7 Hz, H-10′), 5.33 (4H, overlapped, H-8′, -9′, -11′, -12′), 6.07 (1H, t, J=2.2 Hz, H-2), 6.11 (2H, d, J=2.2 Hz, H-4, -6). ^{13}C-NMR (125 MHz, CD_3OD): δ: 14.4 (C-17′), 23.6 (C-16′), 26.5 (C-10′), 28.2 (C-7′, -13′), 30—32 (methylene), 32.7 (C-15′), 37.0 (C-1′), 101.0 (C-2), 107.9 (C-4, -6), 129.03, 129.06, 130.90, 130.91 (C-8′, -9′, -11′, -12′), 146.3 (C-5), 159.3 (C-1, -3).

Demethoxybergenin (10) Colorless needles, mp 305 °C (decomp., MeOH). $[\alpha]_D^{20}$: −22.7° (c=0.08, MeOH). UV λ_{max} (MeOH) nm (log ε): 263 (3.47), 321 (3.16). IR (KBr) cm^{-1}: 3377, 1718, 1340, 1107. HR FAB-MS *m/z*: 299.0751 $[M+H]^+$ (Calcd. for $C_{13}H_{15}O_8$: 299.0767). ^{1}H-NMR (500 MHz, CD_3OD): δ: 3.41 (1H, t-like, J=9.2 Hz, H-3), 3.64 (1H, m, H-2), 3.66 (1H, m, H-11), 3.80 (1H, t-like, J=9.5 Hz, H-4), 4.00 (1H, d, J=9.8 Hz, H-11), 4.04 (1H, dd, J=10.4, 9.5 Hz, H-4a), 4.91 (1H, d, J=10.4 Hz, H-10b), 6.52 (1H, d, J=2.4 Hz, H-9), 7.08 (1H, d, J=2.4 Hz, H-7). ^{13}C-NMR (125 MHz, CD_3OD): δ: 62.7 (C-11), 71.9 (C-3), 74.2 (C-10b), 75.6 (C-4), 81.4 (C-4a), 83.1 (C-2), 110.0 (C-7), 110.4 (C-9), 115.6 (C-10a), 126.1 (C-6a), 157.4 (C-10), 160.3 (C-8), 165.8 (C-6).

DPPH Radical Scavenging Activity DPPH reagent was prepared at 80 μg/ml in MeOH in TLC autographic assay. A test sample (50 μl) was dissolved in EtOH or DMSO and mixed with 100 mM Tris–HCl buffer (pH 7.4, 50 μl), distilled water (50 μl) and 400 μM DPPH ethanolic solution (50 μl). The mixture was shaken well and allowed to stand for 20 min in the dark. The absorbance at 515 nm was measured by a microtiter plate reader (Biorad, model 550). Vitamin E derivative Trolox was used as a positive control. The decrease in the absorbance/μM of each sample was compared with that of Trolox.

Cytotoxicity A cytotoxicity assay against the murine breast cancer cell line, FM3A, was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method.[23] Cells (1×10^4 cells/ml) were incubated for 3 d at 37 °C in 5% CO_2 atmosphere with or without samples. The sample was dissolved in EtOH or DMSO and the final organic solvent concentration in the medium was less than 1%. The cells were treated by MTT solution (5 mg/ml) and further incubated for 4 h. The formed MTT formazan was dissolved by 0.04 N HCl–isopropanol, and the absorbance at 570 nm (reference at 630 nm) was measured by a microtiter plate reader (Biorad, model 550).

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