Ardisiphenols A—C, Novel Antioxidants from the Fruits of *Ardisia colorata*

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Novel alk(en)ylphenols, named ardisiphenols A—C (1—3) were isolated from the fruits of *Ardisia colorata*, together with known alk(en)ylresorcinols (4—6). Their structures were determined by the NMR and MS/MS analyses. All compounds showed scavenging activities towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and cytotoxicities against murine breast cancer cell line, FM3A.

Key words *Ardisia colorata*; Myrsinaceae; ardisiphenol; alk(en)ylphenol; cytotoxicity

Ardisia colorata ROXB. (Myrsinaceae) is used as a folk medicine for liver disease, cough and diarrhoea in Thailand. A previous phytochemical investigation on this species resulted in the isolation of rapanone and ilexol.[1)] No further detailed study has been reported. In the course of the screening for antioxidants in Thai medicinal plants, *A. colorata* showed a scavenging activity towards DPPH radical in TLC autographic assay.[2)] This paper deals with the structure elucidation of the newly isolated alk(en)ylphenols and known alk(en)ylresorcinols, and their cytotoxicities against murine breast cancer cell line, FM3A.

The 75% ethanol extract of the dried fruits of *A. colorata* (500 g) was partitioned by successive extraction with organic solvents of increasing polarity. Initially the attention was paid to the antioxidative compounds in *n*-hexane fraction, then repeated silica gel chromatographies ($CHCl_3$–MeOH, *n*-hexane–AcOEt) and HPLC (MeOH–0.2% AcOH, 85 : 15) were performed to give new compounds **1**—**3** (0.043, 0.084, 0.058%, respectively) and known compounds **4**—**6** (0.022, 0.053, 0.037%, respectively). The six compounds were all positive for ferric chloride test and showed scavenging activities towards DPPH radical in TLC autographic assay.

Ardisiphenol A (**1**) has a molecular formula $C_{23}H_{38}O_4$ 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 of **1** showed signals for tetra-substituted benzene at δ 131.6, 150.6, 102.4, 156.5, 107.9 and 137.4, and signals for an alkyl side chain which were assigned as shown in Fig. 1 by two-dimensional (2D)-NMR spectra. Signals of acetoxyl group were also observed at δ 2.25 (3H, s), 20.5 ($OCO\underline{C}H_3$) and 171.6 ($O\underline{C}OCH_3$). Electron impact (EI)-MS showed a base peak at *m/z* 139 corresponding to a benzylic fragmentation of deacetylated ion of **1** and an intense peak of deacetylated ion of **1** at 335 $(M-Ac)^+$. Therefore, it was suspected that **1** was a benzene derivative tetra-substituted by two hydroxyl groups, an acetoxyl group and an unbranched C_{15} alkyl chain. However, the position of the acetoxyl group was not deduced from above experimental data and the nuclear Overhauser effect (NOE) enhancement was not observed between the acetyl group and any other protons. To locate hydroxyl groups on the benzene ring, the ^{13}C-NMR of **1** was carefully measured in CD_3OH and CD_3OD, respectively. The deuterium isotope effects[3—5)] were observed at 2-(0.10 ppm) and 4-positions (0.10 ppm) when measured in each solvent. The shift observed for the 3-position (0.18 ppm) were thought to be due to the γ-effects of the two vicinal hydroxyl groups. Furthermore, the ^{13}C-NMR chemical shifts of **1** were calculated using ^{13}C substituent chemical shifts (SCS)[6)] for mono-substituted benzene. Calculated chemical shifts for 6-alkyl-1,2,4-trihydroxybenzene-1-*O*-acetate (not shown) was close to the observed ^{13}C-NMR data. Finally, the structure of **1** was determined as shown in Fig. 1.

Ardisiphenol B (**2**) showed a similar pattern of the ^{1}H- and ^{13}C-NMR spectra to those of **1** except for a set of olefin signals [δ 5.33 (2H, m, H-8′, -9′), 130.81, 130.86 (C-8′, -9′)] and allylic methylene signals [δ 2.02 (4H, m, H-7′, -10′), 28.13, 28.16 (C-7′, -10′)]. Molecular formula of **2**, $C_{23}H_{36}O_4$ that was two protons less than **1**, also indicated the existence of a C_{15} monounsaturated alkenyl chain. 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 and its 8′*E* isomer.[7)] To determine the position of the double bond, the negative FAB-MS/MS was applied on the precursor ion at *m/z* 333 $[(M-Ac)^-]$. The observed ions at *m/z* 262 $[(M-Ac-C_5H_{11})^-]$ and 207 $[(M-Ac-C_9H_{17}-H)^-]$ are proposed to result from the allylic cleavages of the side chain. Therefore, the structure of **2** was proposed to be 6-(8′*Z*-pentadecenyl)-1,2,4-trihydroxy-

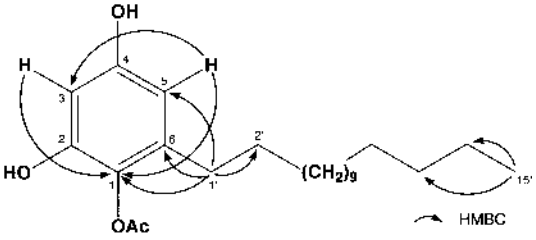

Fig. 1. HMBC Correlations of **1**

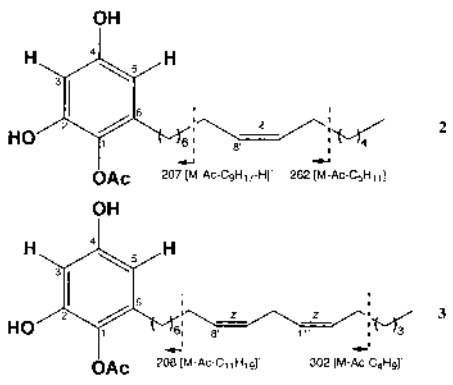

Fig. 2. Fragment Ions Observed in the Negative FAB-MS/MS Spectra of **2** and **3**

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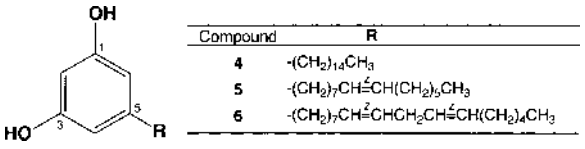

Fig. 3. Structures of the Known Alk(en)ylresorcinols (**4**—**6**)

benzene-1-*O*-acetate (Fig. 2).

The ^{1}H- and ^{13}C-NMR spectra of ardisiphenol C (**3**) revealed the same phenolic subunit as **1** and **2**, but the side chain was C_{17} containing diolefinic structure by a molecular formula $C_{25}H_{38}O_4$ obtained from HR-FAB-MS. The side chain of **3** has the same diene as that of 3-(8′*Z*,11′*Z*-heptadecadienyl)-1,2-dimethoxybenzene[7] from the consideration of the NMR data. Bisallylic methylene signal at δ 2.75 (2H, t, J=6.7 Hz, H-10′) and 25.6 (C-10′) further confirmed that two double bonds were separated by a methylene. The negative FAB-MS/MS applied on the precursor ion at *m/z* 359 [(M−Ac)$^-$] showed the ions at *m/z* 302 [(M−Ac−C_4H_9)$^-$] and 208 [(M−Ac−$C_{11}H_{19}$)$^-$], resulting from the allylic cleavages of the side chain. Thus, the above data led to propose the structure of **3** to be 6-(8′*Z*,11′*Z*-heptadecadienyl)-1,2,4-tetrahydroxybenzene-1-*O*-acetate (Fig. 2).

Compounds **4** and **5** were identified as 5-pentadecyl-1,3-benzenediol[8] and its 8′*Z*-enyl congener,[8] respectively, by spectroscopic methods (Fig. 3). Compound **6** was identical with 5-(8′*Z*,11′*Z*-heptadecadienyl)-1,3-benzenediol[9] (Fig. 3). These compounds were known as alk(en)ylresorcinols and often found in plants of the families Ginkgoaceae, Anacardiaceae, Gramineae and Proteaceae.

The newly isolated ardisiphenol A—C (**1**—**3**)[10] showed potent cytotoxicities against FM3A cells (IC_{50}: 0.7, 0.6, 0.5 μM, respectively), while **4**—**6** have moderate cytotoxicities (IC_{50}: 2.5, 1.8, 2.4 μM, respectively). The presence of the acetoxyl group in ardisiphenols might be important for this activity.

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10) Ardisiphenol A (**1**) (6-pentadecyl-1,2,4-trihydroxybenzene-1-*O*-acetate): Red oil, positive HR-FAB-MS *m/z*: 417.2390 (M+K)$^+$ (Calcd for $C_{23}H_{38}O_4K$: 417.2407). EI-MS *m/z*: 378 (M)$^+$, 335 (M−Ac)$^+$, 139. IR (KBr) cm^{-1}: 3363 (OH), 2922, 2852, 1718, 1238, 1184. UV λ_{max} (MeOH) nm (log ε): 224 (3.85), 279 (3.44). ^{1}H-NMR (500 MHz, CD_3OD): δ 0.89 (3H, t, J=7.0 Hz, 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<u>C</u>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<u>C</u>OCH$_3$).

Ardisiphenol B (**2**) (6-(8′*Z*-pentadecenyl)-1,2,4-trihydroxybenzene-1-*O*-acetate): Red oil, 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 on 333 (M−Ac)$^-$ ion] *m/z*: 333 (M−Ac)$^-$ (100%), 262 (M−Ac−C_5H_{11})$^-$ (0.57%), 207 (M−Ac−C_9H_{17}−H)$^-$ (0.34%). IR (KBr) cm^{-1}: 3393 (OH), 2925, 2854, 1735, 1248, 1184. UV λ_{max} (MeOH) nm (log ε): 224 (3.85), 279 (3.39). ^{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, m, H-7′, -10′), 2.25 (3H, s, Ac), 2.35 (2H, t, J=7.3 Hz, H-1′), 5.33 (2H, m, H-8′, -9′), 6.13 (1H, d, J=2.8 Hz, H-5), 6.21 (1H, d, J=2.8 Hz, H-3). ^{13}C-NMR (125 MHz, CD_3OD): δ 14.4 (C-15′), 20.6 (OCO<u>C</u>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<u>C</u>OCH$_3$).

Ardisiphenol C (**3**) (6-(8′*Z*,11′*Z*-heptadecadienyl)-1,2,4-trihydroxybenzene-1-*O*-acetate): Red oil, 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 on 359 (M−Ac)$^-$ ion] *m/z*: 359 (M−Ac)$^-$ (100%), 302 (M−Ac−C_4H_9)$^-$ (6.42%), 208 (M−Ac−$C_{11}H_{19}$)$^-$ (1.8%). IR (KBr) cm^{-1}: 3398 (OH), 2926, 2855, 1749, 1239, 1183. UV λ_{max} (MeOH) nm (log ε): 223 (3.89), 280 (3.45). ^{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, m, 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, m, H-8′, -9′, -11′, -12′), 6.18 (2H, br s, H-3, -5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.1 (C-17′), 20.6 (OCO<u>C</u>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<u>C</u>OCH$_3$).