Naturally Occurring 5-Lipoxygenase Inhibitor. II. 1)
Structures and Syntheses of Ardisianones A and B, and Maesanin, Alkenyl-1,4-benzoquinones from the Rhizome of Ardisia japonica

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New alkenyl-1,4-benzoquinones, ardisianones A (1) and B (2), and the known maesanin (3) as 5-lipoxygenase inhibitors have been isolated from the rhizome of Ardisia japonica. Their structures have been elucidated as 2-methoxy-6-[(Z)-10′-pentadeceny]-1,4-benzoquinone and 5-hydroxy-2-methoxy-6-[(Z)-8′-trideceny]-1,4-benzoquinone, respectively, on the basis of spectroscopic data and chemical degradation. Ardisianone A (1), maesanin (3) and belamcandol A (7) have been synthesized starting from belamcandol B (6), readily prepared by Wittig reaction between 9-(2-tetrahydropryanloxy)nonanen and 3,5-dimethoxybenzyltriphenylphosphonium bromide followed by selective demethylation with sodium thioethoxide.

Keywords ardisianone A; ardisianone B; maesanin; belamcandol A; belamcandol B; 1,4-benzoquinone

Arsidia japonica (Myrsinaceae) has been used in Chinese traditional medicine as an antitussive, diuretic, and alexipharmic agent, and elaborates phenols such as bergenin and ardisinol, and 1,4-benzoquinones like rapanone and embelin along with a mixture of several alkyl and alkenyl benzoquinones. Among the constituents, only bergenin was identified as an antitussive principle in this plant. In the course of our investigation on prostaglandin biosynthesis regulators in natural products, we found that the n-hexane extract of A. japonica could inhibit specifically 5-lipoxygenase in the cytosol of guinea pig polymorphonuclear leukocytes. Hence, extensive fractionation monitored by inhibitory activity against 5-lipoxygenase resulted in the isolation of two new 1,4-benzoquinones 1 and 2, named ardisianones A and B, respectively, along with maesanin (3), previously isolated as a host defense stimulant from the African medicinal plant Maesa lanceolata. 7,8 Synthesis of ardisianone A (1), maesanin (3), and belamcandol A (7) were also attempted from a common biosynthetic precursor, belamcandol B (6), because of the similarity of their structures and intriguing biological property. In this paper, we deal with the structural elucidation of new benzoquinones 1 and 2, and a degree of 5-lipoxygenase inhibitory activity for all the benzoquinones isolated in this study, as well as with convergent synthesis of compounds 1, 3, 6, and 7.

Figure 1

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a long alkenyl side chain at \( \delta 0.93 \) (3H, t, \( J=7.3 \text{ Hz} \)), 1.20–1.40 (18H, m), 2.01 (4H, m), and 5.35 (2H, t, \( J=4.5 \text{ Hz} \)). The \( ^{13} \text{C} \) nuclear magnetic resonance (\( ^{13} \text{C}-\text{NMR} \)) spectrum showed the signals indicative of a methoxylated 1,4-benzoquinone, at \( \delta 187.5 \) (s, C-4), 182.2 (s, C-1), 158.9 (s, C-2), 147.9 (s, C-6), 133.1 (d, C-5), 107.3 (d, C-3), and 56.2 (q, OCH\(_3\)) and signals at \( \delta 130.0 \) (d, C-10, 11) for the double bond, and at \( \delta 13.9–32.0 \) (13 carbons) for the long side chain. These spectral data disclosed that ardisianone A was a 1,4-benzoquinone substituted at C-2 and 6 with a methoxy group and a long side chain. This was also supported by detection of a base ion peak at \( m/z \) 154 in the mass spectrum (MS) accounting for rupture of the benzyl bond of the alkyl group, and by observation of nuclear Overhauser effects (NOEs) for the H-3 signal at \( \delta 5.88 \) and the H-5 signal at \( \delta 6.48 \) upon irradiation of the methoxy proton signal and the H-1' signal at \( \delta 2.43 \), respectively. The double bond in the side chain was determined to be placed at C-10' and 11' by the identification of the aldehyde 4 (\( m/z \) 292.1665 (M\(^+\)); Calcd 292.1675 for \( \text{C}_{19}\text{H}_{23}\text{O}_4\)). obtained by epoxidation of 1 followed by \( \text{HIO}_4 \) oxidation,\(^{13} \) and its stereochemistry was assigned as Z on the basis of the chemical shift values (\( \delta 27.0 \) and 27.3)\(^{10} \) of the two allylic methylene carbons. These results established ardisianone A (I) to be 2-methoxy-6-[[\( Z \)-10'-pentadecenyl]-1,4-benzoquinone.

Ardisianone B (2), mp 62–64°C, had the molecular formula \( \text{C}_{20}\text{H}_{24}\text{O}_4 \) established by the high resolution electron impact mass spectrum (HREIMS). The UV spectrum showed a dioxygenated benzoquinone chromophore at 287 and 420 nm,\(^{11} \) whereas the IR spectrum displayed the absorptions at 3360 cm\(^{-1}\) and 1660, 1635, and 1600 cm\(^{-1}\) attributable to a hydroxy group and a benzoquinone moiety, respectively. The \( ^1 \text{H}-\text{NMR} \) spectrum contained a singlet signal at \( \delta 5.83 \) which showed NOE interaction with the methoxy proton signal at \( \delta 3.85 \), and a series of signals assignable to a tridecyl group. These spectral data suggest that 2 is closely related to maesain (3). In fact, the \( ^{13} \text{C}-\text{NMR} \) data for the 1,4-benzoquinone ring in 2 were almost identical with those of maesain; 2 (maesain): \( \delta 181.6 \) (181.6, C-1), 161.2 (161.2, C-2), 102.2 (102.2, C-3), 182.8 (182.5, C-4), 151.5 (151.8, C-5), 119.3 (119.6, C-6). On the other hand, the mass spectra showed a slight difference between these compounds, indicating a variant in the position of the double bond in the long side chain. Hence, according to essentially the same procedures used for 1, the position and geometry of the double bond in the tridecyl group was established to be at C-8' and to be Z by oxidative degradation of O-methyl derivative, giving rise to an aldehyde 5 (\( m/z \) 294) and the \( ^{13} \text{C}-\text{NMR} \) shift values (\( \delta 26.9 \), 27.2) for all allylic methylene carbons, respectively. Accordingly, ardisianone B (2) was assigned as 5-hydroxy-2-methoxy-6-[[\( Z \)-8'-trideceny1]-1,4-benzoquinone.

Inhibitory activity against 5-lipoxygenase in the cytosol of guinea pig polymorphonuclear leukocytes\(^{12} \) by the 1,4-benzoquinones isolated in the present study are summarized in Table I. The degree of inhibition caused by each compound was about ten times weaker than AA-861,\(^{13} \) a reference 1,4-benzoquinone.

**Syntheses of Ardisianone A (1), Maesain (3), and Belamcandols A (7) and B (6)** Several synthetic challenges for this type of 1,4-benzoquinones having alkyl chains of various length have been achieved in the past because of their interesting biological activities.\(^{14} \) Most synthetic routes have been focused on elaboration of the side chain on the aromatic ring\(^{8,15–18} \) except for Danheiser's unique

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<th>Table I. Inhibition of 5-Lipoxygenase in the Cytosol of Guinea Pig Polymorphonuclear Leukocytes by Compounds 1–3</th>
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<td><strong>Compound</strong></td>
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\( \text{IC}_{50} \) 0.18 µM.

Fig. 2. Synthetic Scheme for Ardisianone A (I)
method. We envisioned that belamcandol B (6) would function as a common intermediate for our target benzoquinones bearing a (Z)-8-pentadecenyl side chain because related compounds 1, 3, and 7 could presumably be biosynthesized via oxidation from belamcandol B (6). Therefore, first of all, a practical method for the synthesis of belamcandol B (6) has been explored starting from the known phosphonium salt 9.

The phosphonium salt 9 was converted into its ylide and then allowed to react with 9-(2-tetrahydropyranoyloxy)-nonanal (8) which, in turn, was prepared from 9-decen-1-ol by ozonolysis of the double bond after protection of the hydroxy group. The double bond in 10 was reduced by catalytic hydrogenation to give compound 11, the tetrahydropyranyl ether of which was deprotected with aqueous acid hydrolysis followed by Swern oxidation to afford the aldehyde 12 in good yield. Wittig olefination of 12 with n-pentyltriphenylphosphonium iodide gave (Z)-olefin 13 in 93% yield. Selective demethylation of the symmetrical O-methyl groups in 13 was realized with C5H5SnNa in N,N-dimethylformamide (DMF), thus giving belamcandol B (6) in 91% yield.

Compound 6 was easily oxidized into a 1,4-benzoquinone, ardisianone A (1) by a power of molecular oxygen catalyzed by salcomine in 63% yield. The synthetic ardisianone A thereby obtained was superimposed with the natural one in the spectra data (IR, MS, and 1H- and 13C-NMR).

Next, our attention was given to the synthesis of maesanein (3) from the common intermediate 6, which requires selective oxidation at C-5 to achieve our goal. To this end, selective bromination at the C-5 position should be appropriate because it is regarded as equivalent to a requisite hydroxy group. We employed a 3-N-bormusocinumide (NBS) DMF reagent for the selective bromination on the aromatic ring without concomitant bromination of the double bond in the side chain. In spite of a good conversion yield, selectivity (14/15 = 36/55) was not satisfactory. After several attempts, carbon tetrachloride (CCl4) was found to be a suitable solvent to increase selectivity. Bromination of 6 with NBS in CCl4 at room temperature afforded 14 (63%) and 15 (13%), which were readily separable by chromatography on silica gel. The brominated phenol 14 was subjected to the salcomine-catalyzed oxidation, thus furnishing the benzooquinone 16 in 31% yield (75%, based on the reacted 14). Subsequent oxidation at the C-5 position was realized by CH3OH–Na2CO3 in the presence of a catalytic amount of Pd(0) to give the dimethoxybenzoquinone 17 in quantitative yield. It should be noted that the substitution of the bromine with methanol did not proceed in the absence of Pd(0). Finally, a more hindered methoxy group in 17 was selectively hydrolyzed with 70% HClO4 to give maesanein (3) in high yield, which was identical in all respects to the natural one.

Belamcandol A (7) could routinely be derived from ardisianone A (1) by reduction followed by selective methylation as shown in Fig. 4.

In conclusion, we synthesized 5-lipoxygenase inhibitors, ardisianone A (1), maesanein (3), and Belamcandol A (7) from a common biogenetic intermediate, phenol 6, and thus our preparative method of synthesis should allow the ready preparation of analogues such as ardisianone B (2), iruquinone, and iruquinin, which differ primarily in the presence of a hydroxy group at the C-5 position and in the type of side chain attached at the C-6 ring position on the 1,4-benzoquinone nucleus.

**Experimental**

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded on a Hitachi 340 spectrophotometer. IR spectra were measured with a Jasco A-202 spectrophotometer. 1H- and 13C-NMR spectra were obtained at 400 or 200 MHz (1H-NMR) and 100.16 MHz (13C-NMR) using a Bruker WH 400 and a Varian UNITY 200. Chemical shift values were expressed in ppm downfield from tetramethylsiline as an internal standard. The MS were recorded on a Varian MAT 200 and a JEOL JMS SX-102. Silica gel (Wako, C-300) and Sephadex (Pharmacia Fine Chemicals, LH-20) were used for column chromatography. High performance liquid chromatography (HPLC) was performed using a Waters 6000A and a Jasco UV-VIS-110-II UV detector. Both silica gel F254 and RP8 F254 (Merck) were used for analytical thin layer chromatography, and spots were visualized using UV (254 nm) illumination and by spraying 40% CeO2·H2SO4 followed by heating.

**Extraction and Purification**

Dried and powdered rhizomes (2.19 kg) of *Ardisia japonica* collected in Osasa, Tokushima prefecture, were immersed three times in methanol at room temperature for 5 d. Combined methanol extracts were evaporated in vacuo to give a gummy extract, which was partitioned between n-hexane and water–methanol (4:1). The n-hexane soluble portion (9.3 g) was chromatographed over silica gel eluting with CHCl3 and CHCl3–CH3OH (20:1). The fraction eluted with CHCl3 was rechromatographed on silica gel with CHCl3 and then purified by HPLC [column: 20% AgNO3·SiO2, i.d. 8 × 300 mm; solvent: n-hexane–EtOAc (17:3), 3.5 ml/min] to give ardisianone A (1) (58 mg). The fraction eluted with CHCl3–CH3OH (20:1) was rechromatographed on silica gel (CHCl3) and then purified by HPLC [column: Lichtsorob RP-2, i.d. 8 × 300 mm; solvent: CH3OH–water–AcOH (75:25:0.6 ml/min)] to give belamcandol A (2) (98 mg) and maesanein (3) (98 mg).

**Ardisianone A (1)** Yellow needles [from CH3OH–water (1:4), mp 39.5–41.5°C. HREIMS *m/z* 346.2497 (M+). Calcd 346.2507 for C18H12O4. EI-MS *m/z* (rel. int.): 346 (25, M+), 154 (100). UV *λmax* (nm) (ε): 266 (11000), 262 (720), IR *νmax* (cm–1): 2960, 2875, 1680, 1650, 1625, 1605. 1H-NMR (400 MHz, CDCl3, δ): 0.93 (3H, t, J = 7.3 Hz, H-2), 1.20–1.40 (18H, m), 2.01 (4H, m, H-9, 12), 2.43 (2H, dd, J = 7.8, 1.4 Hz, H-8), 3.70 (3H, s, H-15), 4.80 (2H, s, H-10).
13.1-Dimethoxy-5-[Z]-10-pentadecenoyl]benzene (13) To a suspension of n-pentyltritylphenylphosphonium iodide (4.7 g, 11.4 mmol) in THF (20 mL) at room temperature under an argon atmosphere was added a solution of tert-BuOK (1.3 g, 11.4 mmol) in THF (10 mL). After being stirred for 30 min, a solution of 2.6 g of 12 (2.9 mmol) in THF (10 mL) was added to the mixture, and the mixture was stirred for 1 h. Water was added and the mixture was extruded with ether. The organic layer was washed with water, sat. NaHCO₃ sol., dried over MgSO₄, and evaporated to leave a residue, which was chromatographed on silica gel (n-hexane-ethyl acetate: 1:1) to afford 13 (2.17 g, 93%) as an oil. HREIMS m/z: 346.2866 (M⁺). <br>C₄₀H₇₂O₂ (404.5681) calcld: 2.24 (ZH, td, J = 6.0, 1.8 Hz), 2.94 (ZH, J = 7.6, 0.8 Hz, 3.76 (ZH, s), 5.29 (ZH, s), 5.35 (ZH, t, J = 6.7 Hz, 6.23 (ZH, dd, J = 2.3, 3.2 Hz), 6.52 (ZH, dd, J = 2.3, 3.2 Hz).<br><br>2-Methoxy-5-[Z]-10-pentadecenoyl]benzene (14) Int to a stirred mixture of 6 (0.1 g, 0.31 mmol) and salomine (10 mg) in DMF (2 mL) was bubbled oxygen for 14 h. The mixture was poured onto ice water and was extracted with ether. The organic layer was washed with sat. NaCl sol., dried over MgSO₄, and evaporated to dryness. The resulting residue was chromatographed on silica gel to afford 14 (0.7 g, 62%) as yellow needles, mp 40–41°C. Its spectral data (IR, MS, H- and 13C-NMR) were identical with those of aridisione A (1).<br><br>Bromination of 6 with NBS A solution of 6 (0.1 g, 0.31 mmol) and NBS (55 mg, 0.31) in CCl₄ (2 mL) was stirred at room temperature for 4 h. After filtering the precipitates formed, the filtrate was evaporated to dryness to afford the residue which was chromatographed on EoCac. The organic layer was washed with water and with sat. NaCl sol., dried over MgSO₄, and evaporated to leave a residue, which was chromatographed on silica gel (n-hexane-ethyl acetate: 10:1) to afford 14 (0.8 mg, 62%) and 15 (10.5%, 12.9%). HREIMS m/z: 410.1788 (M⁺).<br>C₄₀H₇₂O₂Br (404.5681) calcld: 410.1820 for C₄₀H₇₂O₂Br. EIMS m/z (rel. int): 412 (42) and 410 (42, M⁺), 331 (36), 218 (217), 216 (100). IR νmax cm⁻¹: 3512 (OH). <br>HREIMS m/z: 410.1820 (M⁺), 331.19 (M⁺), 218.0 (M⁺), 216.0 (M⁺).<br>C₄₀H₇₂O₂Br (404.5681) calcld: 410.1820 for C₄₀H₇₂O₂Br. EIMS m/z (rel. int): 412 (42) and 410 (42, M⁺), 331 (36), 218 (217), 216 (100). IR νmax cm⁻¹: 3512 (OH). <br>HREIMS m/z: 410.1820 (M⁺), 331.19 (M⁺), 218.0 (M⁺), 216.0 (M⁺).
Into a stirred solution of 14 (318 mg) and salconime (31 mg) in DMF (5 ml) was bubbled oxygen at room temperature for 20 h. Water was added and the mixture was extracted with ether. The organic layer was washed with sat. NaCl sol., dried over MgSO₄, and evaporated to give a crude product, which was chromatographed on silica gel (n-hexane-EtOAc, 7:1) to afford 16 (100.6 mg, 31%) and the yellow staring material 14 (188.4 mg, 60%). Yellow prisms (from EtOH), mp 89–91 °C. HR EIMS m/z 424.1631 (M⁺), Calcd 424.1601 for C₂₃H₂₄O₂B. Br. Anal. Calcd for C₂₃H₂₄BrO₂C: 62.11; H: 7.82; Found: C: 62.22; H: 7.99. EIMS m/z (rel. int.): 426 (52) and 424 (47, M⁺) 345 (28), 233 (36), 231 (36), 193 (57), 179 (30), 153 (100). IR νmax cm⁻¹: 1678, 1639, 1628, 1466, 1440. 1H-NMR (200 MHz, CDCl₃): δ 6.89 (3H, t, J = 7.1 Hz), 2.69 (2H, t, J = 7.7 Hz), 3.84 (3H, s), 3.55 (2H, t, J = 4.3 Hz), 6.67 (1H, s).

2.5-Dimethoxy-6-[Z]-10'-pentadecenyl]-1,4-benzoquinone (17) To a stirred solution of 16 (11.0 mg, 0.025 mmol) and tetrakistriphenylphosphine palladium (0.5 mg) in CH₂OH-THF (10 ml, 1:1) under argon was added 2-equiv. Na₂CO₃ sol. (0.05 ml). The mixture was stirred at 90 °C for 10 min. The solvent was evaporated to leave a residue, which was partitioned between EtOAc and water. The EtOAc layer was washed with sat. NaCl sol., dried over MgSO₄, and evaporated to dryness. The residue was chromatographed on silica gel (n-hexane-EtOAc, 5:1) to give 17 (9.5 mg, 97%) as an oil. HR EIMS m/z 276.2594 (M⁺), Calcd 276.2615 for C₂₃H₂₄O₂. EIMS m/z (rel. int.): 376 (100), 362 (4), 169 (22). 1H-NMR (200 MHz, CDCl₃): δ 6.89 (3H, t, J = 7.5 Hz), 2.43 (2H, t, J = 7.3 Hz), 3.80 (3H, s), 4.05 (3H, s), 5.35 (2H, t, J = 3.5 Hz), 5.72 (1H, s).

2-Hydroxy-5-methoxy-3-[Z]-10'-pentadecenyl]-1,4-benzoquinone (Maesamnin) (3) To a solution of 17 (100 mg) in CH₂Cl₂ (3 ml) was added two drops of 70% HClO₄ at room temperature under argon and the mixture was stirred for 2 h. The resulting mixture was washed with sat. NaHCO₃ sol. and sat. NaCl sol., dried over MgSO₄, and evaporated to give a crystalline solid (80 mg). Recrystallization from C₂H₅OH gave 3 as yellow needles, mp 69–70 °C (lit. mp 70 °C), which was identical in the spectral data (IR, MS, and 1H-NMR) with maesamnin (3).

1,4-Dihydroxy-2-methoxy-2-[Z]-10'-pentadecenyl]-benzene (18) To a stirred solution of 1 (67.3 mg, 0.19 mmol) in THF-MeOH-H₂O (10 ml, 4:3:1) at room temperature under argon was added Na₂SO₄ (135.3 mg, 0.76 mmol) and stirring was continued for 2 h. The reaction solution was condensed in vacuo and extracted with EtOAc. The organic layer was washed with water and sat. NaCl sol., dried over MgSO₄, and evaporated to dryness. The residue was chromatographed on silica gel (n-hexane-EtOAc, 5:1) to give 18 (40 mg, 61%). HR EIMS m/z 264.2464 (M⁺), Calcd 264.2465 for C₂₂H₂₃O₂. EIMS m/z: 248 (100, M⁺), 154 (41). 1H νmax cm⁻¹: 3553, 3377, 1606, 1496, 1473, 1440. 1H-NMR (200 MHz, CDCl₃): δ 6.91 (3H, t, J = 7.3 Hz), 2.55 (2H, t, J = 7.3 Hz), 3.83 (3H, s), 4.05 (3H, s), 5.35 (2H, t, J = 4.5 Hz), 6.21 (1H, d, J = 2.2 Hz), 6.32 (1H, d, J = 2.2 Hz).

2.4-Dimethoxy-6-[Z]-10'-pentadecenyl]-phenol (Belcamandal A) (7) A mixture of 18 (13 mg, 0.037 mmol), methyl iodide (0.03 mmol), K₂CO₃ (7.8 mg), and acetone (5 ml) was refluxed for 2 h. After being cooled to room temperature, water was added and the mixture was extracted with EtOAc. The organic layer was washed with water and sat. NaCl sol., dried over MgSO₄, and evaporated to dryness. The residue was purified by preparative TLC (n-hexane-EtOAc, 4:1) to yield 7 (4 mg, 30%), which was identical in 1H-NMR, IR, and MS spectrum with the natural specimen.

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References and Notes
5) L. Bao-Ling and Y. Zan-Xi, Ko Hsueh Tung Pao, 24, 910 (1979) [Chem. Abstr., 92, 72678z (1982)]
9) Ardisianone A was also isolated from Belamanda chinesis; see ref. 1.