Ardisinones A−E, Novel Diarylundecanones from *Ardisia arborescens*

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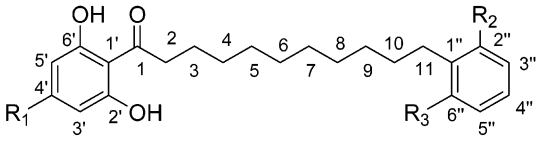
A phytochemical study on an ethanol extract of *Ardisia arborescens* resulted in the isolation of five new diarylundecanones, named ardisinones A−E (**1**−**5**). The structures were established by HRESIMS and NMR (^{1}H, ^{13}C, DEPT, HSQC, HMBC) as 11-(2-acetoxy-6-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-undecan-1-one (**1**), 11-(2-acetoxy-6-hydroxyphenyl)-1-(2,6-dihydroxy-4-methoxyphenyl)undecan-1-one (**2**), 1-(2,6-dihydroxy-4-methoxyphenyl)-11-(2,6-dihydroxyphenyl)undecan-1-one (**3**), 1-(2,4,6-trihydroxyphenyl)-11-(2,6-dihydroxyphenyl)undecan-1-one (**4**), and 1-(2,4,6-trihydroxyphenyl)-11-(2-hydroxyphenyl)undecan-1-one (**5**). In our in vitro disk diffusion assay, compounds **1** and **4** showed some slight inhibition of three bacteria, while **2** and **3** did not show antimicrobial activity.

Ardisia arborescens Wall. Ex A. Dc (Myrsinaceae) is an endemic herbaceous plant distributed in the southwest region of the People's Republic of China. It is used as a febrifuge to relieve internal fevers.[1] No previous phytochemical investigation on this species has been conducted. The medicinal uses of *A. arborescens* prompted us to investigate polar components in the EtOH extract. Five new closely related compounds (**1**−**5**), together with five known compounds, 1-triacontanol,[2] β-sitosterol,[3] β-daucosterol,[3] urs-12-en-3β-ol,[4] and succinic acid,[5] were isolated. The known compounds were identified on the basis of spectral comparison with published data from the literature. Diarylnonoids are rare in nature,[6] especially diarylundecanones, which possess 11 carbon units between two aromatic rings.[7]



Compound	R_1	R_2	R_3
1	OH	OAc	OH
2	OMe	OAc	OH
3	OMe	OH	OH
4	OH	OH	OH
5	OH	OH	H

The molecular formula ($C_{25}H_{32}O_7$) of compound **1** was obtained from the HRESIMS at *m*/*z* 445.2219 $[M + H]^+$. The $FeCl_3$ positive reaction and the IR absorption at 3423 cm^{-1} confirmed the presence of phenolic hydroxyl groups. A bathochromic shift at 281 nm appearing in the UV spectrum with the addition of base or aluminum trichloride, along with an IR absorption at 1630 cm^{-1}, suggested the presence of an *ortho*-hydroxycarbonyl-substituted aryl ring.[8] As evident from the ^{1}H NMR spectrum (Table 1), the five aromatic proton signals [δ 5.78 (2H, s), 6.67 (1H, dd, J = 8.0, 1.5 Hz), 6.97 (1H, m), and 6.46 (1H, dd, J = 8.0, 1.5 Hz)] revealed the existence of two benzene rings, four hydroxyl groups at δ 12.23 (2H, s), 10.31 (1H, s), and 9.53 (1H, s), one acetoxyl group [δ_H 2.24 (3H, s), δ_C 21.2 (OCO*C*H$_3$) and 169.8 (O*C*OCH$_3$)], and an aliphatic chain. Since the ^{1}H NMR and ^{13}C NMR spectra of **1** were similar to those of malabaricones,[6] the presence of 10 methylenes—one flanking the carbonyl group [δ_H 2.95 (2H, t, J = 7.4 Hz), δ_C 43.7], one benzylic [δ_H 2.37 (2H, t, J = 7.4 Hz), δ_C 24.1], and eight others [δ_H 1.55 (2H, q, J = 7.0 Hz), δ_H 1.24 (12H, overlapped), δ_H 1.37 (2H, m), δ_C 25.1, 29.1, 29.5, 29.6 (3C), 29.7 (2C)]—was inferred.[6]

All carbon and proton chemical shift assignments and function group positions were obtained through analysis of DEPT, HSQC, and HMBC experiments. The two protons (δ_H 5.78 s, δ_C 95.3) assigned at 3′ and 5′ by HSQC experiment were characteristic of a symmetrical 1′,2′,4′,6′-tetrasubstituted aryl ring, the structure of which was further confirmed by the HMBC correlations of H-3′/C-1′, C-2′, and C-4′ and of H-5′/C-1′, C-4′, and C-6′. The two hydroxyls located at 2′ and 6′ were confirmed by the HMBC correlations of OH-2′/C-1′, C-2′, and C-3′ and of OH-6′/C-1′, C-5′, and C-6′. Since intermolecular hydrogen bonding with the carbonyl carbon caused the downfield shift of two hydroxyls at 2′ and 6′ rather than the hydroxyl at 4′, the carbonyl carbon was positioned at position 1. Support for this assignment was readily obtained from the EIMS spectrum, which had prominent peaks resulting from cleavage α to the carbonyl group at *m*/*z* 153 (trihydroxylbenzoyl ion) and a peak of high relative intensity at *m*/*z* 168 $[C_6H_3(OH)_3C(OH)=CH_2]^+$.[8] The coupling constants of the aromatic protons in the ^{1}H NMR spectrum suggested the presence of a 1″,2″,6″-trisubstituted benzene unit. The carbon at δ 122 was established at C-1″, as confirmed by a long-range correlation of this carbon with the proton at δ 2.37 attributed to H-11. The hydroxyl group at δ 9.53 was connected to C-6″ by analysis of the HMBC correlations with C-1″ and C-6″. The acetoxyl group was further proved by the HMBC correlation between the carbonyl carbon resonating at δ 169.8 and the proton at δ 2.24 (3H, s). Thus, the structure of compound **1** was deduced to be 11-(2-acetoxy-6-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-undecan-1-one.

The remaining four compounds had UV absorption and ^{1}H and ^{13}C resonances resembling those of **1** (Tables 1 and

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Table 1. ^{1}H NMR Data for Compounds **1**−**5** (DMSO-d_6)

proton	1	2	3	4	5
2	2.95 (2H, t, 7.4)	2.98 (2H, t, 7.3)	2.98 (2H, t, 7.3)	2.95 (2H, t, 7.5)	2.96 (2H, t, 7.3)
3	1.55 (2H, q, 7.0)	1.56 (2H, q, 7.0)	1.56 (2H, q, 7.0)	1.55 (2H, q, 7.0)	1.56 (2H, q, 7.0)
4−9	1.24 (12H, brs)	1.24 (12H, brs)	1.24 (12H, brs)	1.24 (12H, brs)	1.25 (12H, brs)
10	1.37 (2H, m)	1.38 (2H, m)	1.39 (2H, m)	1.39 (2H, m)	1.49 (2H, m)
11	2.37 (2H, t, 7.4)	2.37 (2H, t, 7.3)	2.45 (2H, t, 7.3)	2.45 (2H, t, 7.5)	2.48 (2H, t, 7.8)
3′, 5′	5.78 (2H, s)	5.93 (2H, s)	5.93 (2H, s)	5.78 (2H, s)	5.79 (2H, s)
3″	6.67 (1H, dd, 8.0, 1.5)	6.67 (1H, dd, 8.0, 1.5)	6.22 (1H, d, 8.0)	6.22 (1H, d, 8.0)	6.74 (1H, dd, 8.0, 1.2)
4″	6.97 (1H, m)	6.97 (1H, m)	6.70 (1H, t, 8.0)	6.70 (1H, t, 8.0)	6.96 (1H, m)
5″	6.46 (1H, dd, 8.0, 1.5)	6.46 (1H, dd, 8.0, 1.5)	6.22 (1H, d, 8.0)	6.22 (1H, d, 8.0)	6.68 (1H, m)
6″					7.00 (1H, dd, 7.4, 1.2)
2′, 6′-OH	12.23 (2H, s)	12.31 (2H, s)	12.31 (2H, s)	12.21 (2H, s)	12.22 (2H, s)
4′-OH	10.31 (1H, s)			10.29 (1H, s)	10.30 (1H, s)
4′-OCH_3		3.72 (3H, s)	3.72 (3H, s)		
2″-OH			8.91 (1H, s)	8.88 (1H, s)	9.12 (1H, s)
6″-OH	9.53 (1H, s)	9.53 (1H, s)	8.91 (1H, s)	8.88 (1H, s)	
CH_3	2.24 (3H, s)	2.24 (3H, s)			

Table 2. ^{13}C NMR Data for Compounds **1**−**5** (150 MHz, DMSO-d_6)

carbon	1	2	3	4	5
1	205.9	206.6	206.6	205.9	205.9
2	43.7	43.9	43.9	43.7	43.7
3	25.1	24.9	25.0	25.1	25.1
4	29.1[a]	29.1[a]	29.4[a]	29.4[a]	29.6[a]
5	29.5[a]	29.4[a]	29.5[a]	29.6[a]	29.6[a]
6	29.6[a]	29.4[a]	29.6[a]	29.7[a]	29.6[a]
7	29.6[a]	29.5[a]	29.6[a]	29.7[a]	29.6[a]
8	29.6[a]	29.5[a]	29.7[a]	29.8[a]	29.7[a]
9	29.7[a]	29.6[a]	29.7[a]	29.8[a]	30.0[a]
10	29.7[a]	29.6[a]	29.9[a]	30.0[a]	30.2[a]
11	24.1	24.1	23.4	23.4	25.1
1′	104.4	105.3	105.3	104.4	104.4
2′, 6′	164.8	164.6	164.6	164.8	164.8
3′, 5′	95.3	93.8	93.8	95.3	95.3
4′	165.1	166.0	166.0	165.1	165.1
1″	122.0	122.0	115.9	115.9	129.1
2″	150.4	150.4	156.7	156.8	155.7
3″	113.0	113.0	106.8	106.8	115.5
4″	126.9	127.0	126.5	126.5	127.2
5″	113.6	113.7	106.8	106.8	119.4
6″	157.0	156.9	156.7	156.8	130.3
4′-OCH_3		56.0	56.0		
C═O	169.8	170.0			
CH_3	21.2	21.2			

[a] Values bearing the same superscript in the same column may be interchangeable.

2). It was apparent that they differed only in the substitutions at the two benzene rings. The molecular formula of $C_{26}H_{34}O_7$ for ardisinone B (**2**) was confirmed by high-resolution ESIMS. The ^{1}H NMR spectrum differed from that of ardisinone A (**1**) in having one less hydroxyl signal at the tetrasubstituted aryl ring. Comparison of the ^{13}C NMR chemical shifts of **2** and **1** indicated the hydroxyl group at C-4′ in **1** was substituted by a methoxyl group in **2**, and the change of substitution induced the upfield shifts of C-3′ and C-5′ of compound **2** compared with **1**. Accordingly, the structure of **2** was assigned as 11-(2-acetoxy-6-hydroxyphenyl)-1-(2,6-dihydroxy-4-methoxyphenyl)undecan-1-one.

Ardisinone C (**3**), $C_{24}H_{32}O_6$, had ^{1}H and ^{13}C NMR spectra indicating the same features as **2** except for a hydroxyl (δ 8.91) instead of an acetoxyl group at 2″ of the trisubstituted benzene ring. The symmetrical substitutions at the trisubstituted aryl ring by hydroxyls at C-2″ and C-6″ and the shifts of protons at C-3″ and C-5″ were identical, and the proton at C-4″ displayed one doublet instead of the multiplet of compound **2**. Thus, compound **3** was established as 1-(2,6-dihydroxy-4-methoxyphenyl)-11-(2,6-dihydroxyphenyl)undecan-1-one.

Ardisinone D (**4**), $C_{23}H_{30}O_6$, possessed the same tetrasubstituted aryl ring as **1** and the same oxygenation pattern of the trisubstituted ring as **3**. Thus, compound **4** was characterized as 1-(2,4,6-trihydroxyphenyl)-11-(2,6-dihydroxyphenyl)undecan-1-one.

Ardisinone E (**5**), $C_{23}H_{30}O_5$, showed ^{1}H and ^{13}C NMR spectra similar to those of compound **4** at the tetrasubstituted aryl ring, although a hydroxyl signal less than compound **4** at the other ring. Since there was only one hydroxyl group signal at the other benzene ring, the resonances for C-1″, C-3″, and C-5″ in the ^{13}C NMR shifted downfield compared to compound **4**. Therefore, the structure of compound **5** was 1-(2,4,6-trihydroxyphenyl)-11-(2-hydroxyphenyl)undecan-1-one.

HPLC analysis of compounds **3** and **4** in the ethanolic extract was done to investigate whether compounds **3** and **4** were artifacts derived from compounds **1** and **2**. The components were identified by comparison of their retention times with those of external standards. The presence of **3** and **4** in the chromatogram of the ethanolic extract verified that they were not artifacts formed during the isolation procedure.

Diarylundecanones have been reported to inhibit some common microorganisms.[9] Six microorganism species (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium smegatis*, *Candida albicans*, *Aspergillus niger*) were evaluated for growth inhibition by ardisinones A−D (**1**−**4**). Compounds **1** and **4** showed slight inhibition of Gram-positive *S. aureus* and *B. subtilis* and were active against *M. smegatis*, whereas compounds **2** and **3** were not active.

Experimental Section

General Experimental Procedures. Melting points were determined on a XRC-1 apparatus and are uncorrected. UV spectra were recorded on a Perkin-Elmer Lambda 35 UV/vis spectrometer. IR spectra were measured on a Perkin-Elmer Spectrum One FT-IR spectrometer. 1D- and 2D-NMR spectra were performed on a Bruker Avance 600 instrument. Chemical shift values are in ppm (δ) with TMS as internal standard. Column chromatography (CC) was carried out on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China) and polyamide (200 mesh, Linjiang Chemical Inc., Jiangsu, China). High-performance liquid chromatography (HPLC) was performed using a Perkin-Elmer series 200 pump equipped with a Perkin-Elmer series 200 UV/vis detector. TLC was performed on precoated plates (GF_{254}, Qingdao Marine Chemical Inc., China) with the solvent system petroleum ether−Me_2CO (3:1) (*a*). MS were detected on a Bruker Dalonics Apex II mass spectrometer (HRESIMS), a Finnigan LCQ^{DECA} (ESI), and a VG7070E (EI).

Plant Material. *A. arborescens* was collected from Xishuangbanna, Yunan Province, People's Republic of China, in April 2001, and identified by Prof. Jing-yun Cui. A voucher specimen (No. 20010401) was deposited in the herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences.

Bioassay. The in vitro disk diffusion assay to test antimicrobial activity was conducted as previously described.[10] Six microorganism species (*E. coli, S. aureus, B. subtilis, M. smegatis, C. albicans, A. niger*) were evaluated for growth inhibition by compounds **1**−**4**. Each microorganism was grown on an agar medium. Plates were inoculated by spreading the spore or cell suspension evenly over the surface of the agar medium to obtain confluent growth. The zone of inhibition at each disk was measured at 24 and 48 h.

Extraction and Isolation. Dried and powdered *A. arborescens* (3.5 kg) was extracted with 95% EtOH at room temperature to give an extract (220 g), which was suspended in MeOH (90%)−H_2O (10%) and extracted with petroleum ether. Then the MeOH fraction was evaporated under reduced pressure. The residue suspended in H_2O was extracted with EtOAc and *n*-BuOH successively. The EtOAc extract (23 g) was separated by CC (silica gel, $CHCl_3$−MeOH (50:1, 20:1, 10:1, and 5:1) to yield four fractions. Fraction 1 was subjected to CC (silica gel, petroleum ether−Me_2CO, 5:1) to yield compound **2** (156 mg). Fraction 2 on CC (silica gel, petroleum ether−Me_2CO, 4:1) gave compound **3** (134 mg). Compounds **1** (52 mg) and **5** (8 mg) were obtained from fraction 3 by CC (silica gel, petroleum ether−Me_2CO, 5:2, 5:3). After separation on CC (silica gel, petroleum ether−Me_2CO, 3:1) and purification on CC (polyamide, $CHCl_3$−MeOH, 5:1) fraction 4 gave compound **4** (46 mg). Compound **5** was further purified by CC (silica gel, $CHCl_3$−MeOH, 15:1).

Ardisinone A (1): pale yellow needles ($CHCl_3$); mp 103−104 °C; UV (MeOH) λ_{max} 281, 222 (sh), 202 nm; UV (MeOH + NaOH) λ_{max} 319, 223; UV ($AlCl_3$) λ_{max} 369, 307, 220, 205 nm; IR (KBr) ν_{max} 3423, 1730, 1630, 1603, 1524, 1464, 1239, 1173 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* [M] 444 (15), 402 (5), 334 (11), 276 (36), 168 (18), 153 (100), 123 (85), 107 (11), 98 (4), 55 (12), 43 (33); ESIMS *m/z* 445 $[M + H]^+$, 467 $[M + Na]^+$, 483 $[M + K]^+$, 443 $[M - H]^-$; R_f 0.35 (*a*); HRESIMS *m/z* 445.2219 $[M + H]^+$ (calcd for $C_{25}H_{32}O_7$, 445.2221).

Ardisinone B (2): pale yellow needles ($CHCl_3$); mp 68−69 °C; UV (MeOH) λ_{max} 282, 225 (sh), 201 nm; IR (KBr) ν_{max} 3549, 3370, 1745, 1722, 1630, 1592, 1528, 1464, 1247, 1163 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 459 $[M + H]^+$, 481 $[M + Na]^+$, 496 $[M + K]^+$; 457 $[M - H]^-$; R_f 0.44 (*a*); HRESIMS *m/z* 459.2375 $[M + H]^+$ (calcd for $C_{26}H_{34}O_7$, 459.2377).

Ardisinone C (3): white needles ($CHCl_3$); mp 108−109 °C; UV (MeOH) λ_{max} 282, 223 (sh), 205 nm; IR (KBr) ν_{max} 3368, 1631, 1602, 1517, 1469, 1208, 1164 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 417 $[M + H]^+$, 439 $[M + Na]^+$, 455 $[M + K]^+$, 415 $[M - H]^-$; R_f 0.42 (*a*); HRESIMS *m/z* 417.2269 $[M + H]^+$ (calcd for $C_{24}H_{32}O_6$, 417.2272).

Ardisinone D (4): pale yellow needles ($CHCl_3$); mp 67−68 °C; UV (MeOH) λ_{max} 285, 224 (sh), 204 nm; IR (KBr) ν_{max} 3412, 1627, 1523, 1464 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 403 $[M + H]^+$, 401 $[M - H]^-$; R_f 0.31(*a*); HRESIMS *m/z* 403.2119 $[M + H]^+$ (calcd for $C_{23}H_{30}O_6$, 403.2115).

Ardisinone E (5): white amorphous solid; mp 52−53 °C; UV (MeOH) λ_{max} 283, 224 (sh), 203 nm; IR (KBr) ν_{max} 3401, 1631, 1603, 1523, 1455, 1239 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 387 $[M + H]^+$, 385 $[M - H]^-$; R_f 0.38 (*a*); HRESIMS *m/z* 387.2169 $[M + H]^+$ (calcd for $C_{23}H_{30}O_5$, 387.2166).

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