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New phenylphenalenones from banana fruit

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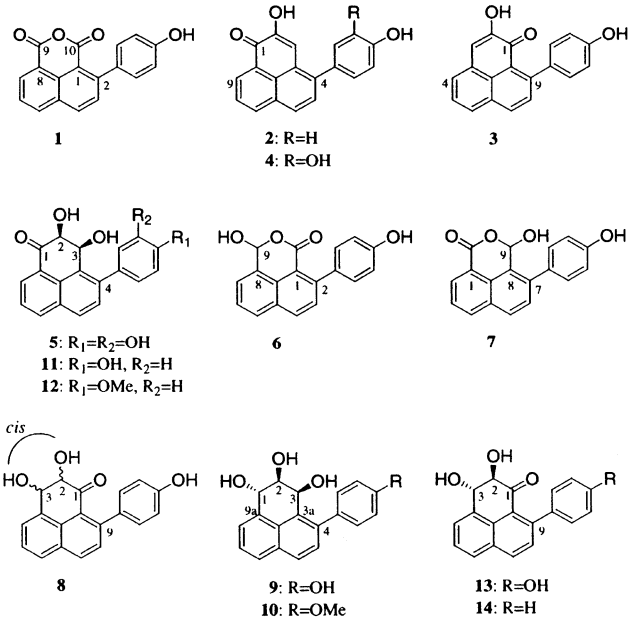
Abstract—Six new phenylphenalenone derivatives were isolated from unripe banana, *Musa acuminata*, fruit that had been wounded followed by inoculation with *Colletotrichum musae*. Absolute configurations of optically active phenylphenalenone derivatives were elucidated by their chemical conversion to L-arabitol pentaacetate. Antifungal activities against *C. musae* and a biosynthetic pathway of phenylphenalenones are also discussed. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Unripe fruit of banana, *Musa acuminata*, produce antifungal compounds upon infection by *Colletotrichum musae*.[1] These compounds are involved in the defense mechanism by which the pathogen remains quiescent until the fruit ripen.[2] We have isolated 17 phenylphenalenone derivatives including 2-(4′-hydroxyphenyl)-1,8-naphthalic anhydride (**1**), irenolone (**2**) and hydroxyanigorufone (**3**) from unripe fruit of *M. acuminata* [AAA] cv. Buñgulan and *M. balbisiana* [BBB] cv. Saba sa Hapon, which were wounded and then inoculated with conidia of *C. musae*.[3–5] Quantitative analysis showed that these compounds were not detected in the healthy tissues and accumulated after wounding and/or inoculation, confirming that they were phytoalexins.[6] On further analysis of extracts from the fruit peel, we found six phenylphenalenone derivatives (**4**–**9**) as novel compounds. Among the six phenylphenalenones, compounds **5**, **8** and **9** are optically active. In addition, known phenylphenalenones from banana, (−)-1,2-*trans*-2,3-*cis*-2,3-dihydro-1,2,3-trihydroxy-4-(4′-methoxyphenyl)phenalene (**10**), (+)-*cis*-2,3-dihydro-2,3-dihydroxy-4-(4′-hydroxyphenyl)phenalene (**11**), (+)-*cis*-2,3-dihydro-2,3-dihydroxy-4-(4′-methoxyphenyl)phenalene (**12**), (−)-*trans*-2,3-dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)-phenalene (**13**) and (−)-*trans*-2,3-dihydro-2,3-dihydroxy-9-phenylphenalene (**14**) are also optically active, but their absolute configurations have not been elucidated.[4,7–10] Determination of their configurations is needed to estimate phenylphenalenone biosynthesis in banana fruit. This paper describes isolation and identification of the new compounds, and the first determination of the absolute configurations of the optically active phenylphenalenone derivatives. An alternative biosynthetic pathway is also discussed based on their structures and the results obtained so far.



2. Results and discussion

Unripe fruit were wounded and then inoculated with a suspension of conidia of *C. musae*. TLC analysis of the extract from the peel revealed the presence of six unidentified spots, and new compounds **4**–**9** corresponding to the spots were isolated. Compound **4** showed signals of a 1,2,4-substituted benzene, a 2,4- or 2,9-substituted phenalene-1-one and three hydroxyl groups in the ^{1}H NMR spectrum, suggesting that **4** was 2-hydroxyphenalen-1-one substituted

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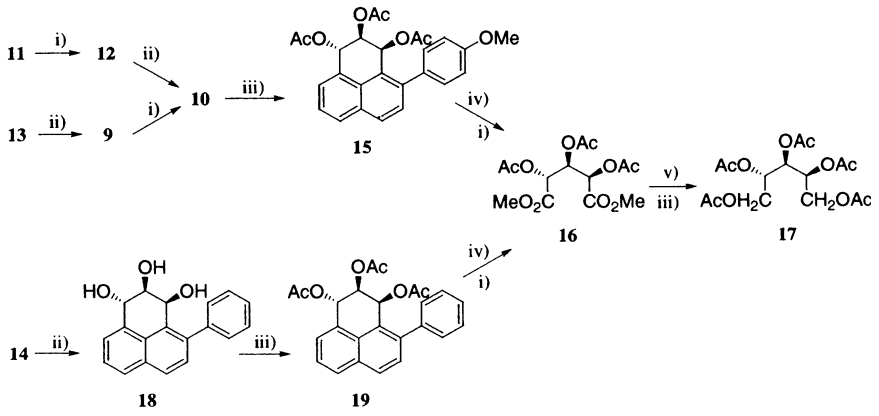


Scheme 1. Determination of absolute configurations of compounds **9**–**14**. (i) CH_2N_2/MeOH; (ii) $NaBH_4$/EtOH (room temperature); (iii) Ac_2O/pyridine; (iv) (a) O_3/AcOH, (b) H_2O_2/AcOH; (v) $NaBH_4$/MeOH (100°C).

at C-4 or C-9 with a 2,4- or 3,4-dihyroxyphenyl group. Signals of three protons on the dihydroxyphenyl group were observed at δ 6.88 (1H, dd, J=8.1, 2.1 Hz), 7.02 (1H, d, J=2.1 Hz), and 7.03 (1H, d, J=8.1 Hz) ppm. These chemical shifts indicated that the phenyl group had two hydroxyl groups not at C-2 and -4 but at C-3 and -4, since in a 2,4-dihydroxyphenyl group, a doublet signal of 3-*H* would have appeared at about δ 6.4 ppm due to the electron-donating effect of 2,4-dihydroxy groups.[11] The position of the dihydroxyphenyl group, C-4 or C-9, was identified by comparison of the relative intensity of the $[M]^+$ ion with that of the $[M-H]^+$ ion in the EI mass spectrum. 4-Phenylphenalenones give lower intensities of $[M-H]^+$ ions than $[M]^+$ ions, whereas 9-phenylphenalenones give higher intensities of $[M-H]^+$ ions than $[M]^+$ ions due to ready formation of stable $[M-H]^+$ ions by bonding between the 1-oxygen and C-2′ followed by elimination of a hydrogen radical at C-2′.[4] Compound **4** gave lower intensity (16%) of the $[M-H]^+$ ion at *m/z* 303 than that (100%) of an $[M]^+$ ion at *m/z* 304 in the mass spectrum, indicating that the side chain was located at C-4. These results showed that **4** was 4-(3′,4′-dihydroxyphenyl)-2-hydroxyphenalen-1-one.

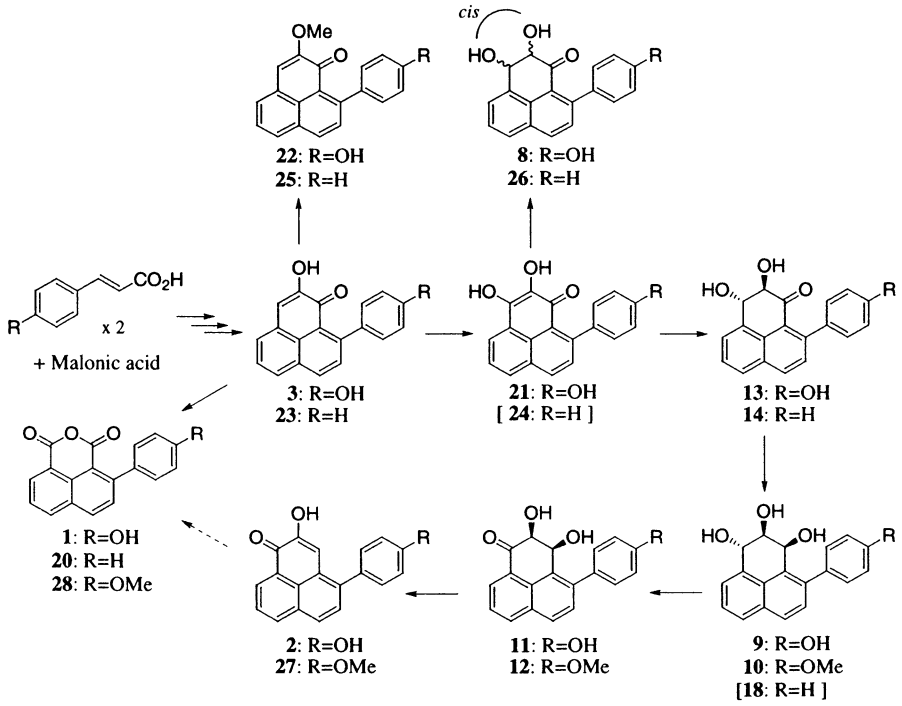
The ^{1}H NMR spectrum of **5** was similar to that of **4**, except for the absence of a singlet assigned to 3-*H* and the presence of two doublets at δ 4.79 and 5.34 ppm (J=3.3 Hz). The doublets were assigned to vicinal protons in a partial structure CX–C*H*(OH)–C*H*(OH)–CX′, in which X and X′ were electron-inducting groups, suggesting that **5** was the 2,3-hydrated derivative of **4**. In its mass spectrum, compound **5** did not give a molecular ion, but gave an ion at *m/z* 304, which would be a dehydrated ion formed by dehydration at C-2 and C-3. The structure presumed was supported by an observation that **5** partly changed to **4** during purification and preservation. The relative configuration between C-2 and C-3 was deduced to be *cis*, due to the J value. These results identified **5** as (+)-2,3-*cis*-4-(3′,4′-dihydroxyphenyl)-2,3-dihydro-2,3-dihydroxyphenalen-1-one. The determination of absolute configurations of C-2 and C-3 will be discussed later.

The ^{1}H NMR spectrum of **6** was similar to that of **1**, except for the presence of two singlet signals (1*H*, respectively) at δ 6.89 and 7.02 ppm.[3] The latter signal disappeared following addition of deuterated water, while the former did not, indicating that the latter signal was due to a hydroxyl proton. The mass spectrum of **6** showed an $[M]^+$ ion at *m/z* 292, which was equal to the molecular weight of a 9- or 10-dihydro derivative of **1**. The doublet corresponding to 4-*H* (8.24 ppm) appeared in a magnetic field lower than those corresponding to 5-*H* (7.78 ppm) and 7-*H* (8.07 ppm) in the ^{1}H NMR spectrum. This would be due to the electron-attracting effect of the carbonyl group at C-10, indicating that **6** is the 9-dihydro derivative of **1**. Thus, compound **6** was identified as 2-(4′-hydroxyphenyl)naphthal-8-formyl-1-carboxylic anhydride. Compound **6** was an optically inactive hemiacetal, indicating that it was a racemic mixture. Racemization could be achieved easily via the aldehyde form.

The ^{1}H NMR spectrum of **7** was similar to that of **6**, but the doublets corresponding to 2-*H* (8.40 ppm) and 4-*H* (8.33 ppm) appeared in a magnetic field lower than that of 5-*H* (8.12 ppm) in the ^{1}H NMR spectrum. The molecular weight of **7** was the same as that of **6**. These observations indicated that **7** was the 10-dihydro derivative of **1**, 7-(4′-hydroxyphenyl)naphthal-8-formyl-1-carboxylic anhydride. Compound **7** was also a racemic mixture. In banana fruit, compounds **6** and **7** might be precursors of **1** since oxidation of **6** and **7** at C-9 gave **1**.

Compound **8** seemed to be an isomer of **13**, since the ^{1}H NMR spectrum of **8** showed signals similar to that of **13** except for the J value between 2-*H* and 3-*H*. The J value, 3.7 Hz, suggested that 2-*H* and 3-*H* were *cis* in **8** but *trans* (J=10.0 Hz) in **13**. In the mass spectrum of **8**, an $[M]^+$ ion was observed at *m/z* 306, similarly to **13**. These results showed that **8** was (−)-*cis*-2,3-dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)phenalen-1-one.

The ^{1}H NMR spectrum of **9** was similar to that of **10**, except for the absence of a singlet signal of a methoxy group at δ 3.89 ppm, suggesting that **9** was the demethyl form of **10**.[7] The mass spectrum of **9** showed an $[M]^+$ ion at *m/z* 308, consistent with the molecular weight of the demethyl form of **10**. The relative configurations of **9** between C-1 and C-2, and C-2 and C-3 were deduced to be *trans* and *cis*, respectively, by the J values (9.8 and 2.7 Hz) observed, the same as **10**. These results indicated that **9** was (−)-1,2-*trans*-2,3-*cis*-2,3-dihydro-1,2,3-trihydroxy-4-(4′-hydroxyphenyl)-phenalene.



Scheme 2. A proposed pathway of phenylphenalenone biosynthesis in banana fruit. Compounds **18** and **24** have not been found in banana.

Elucidation of the absolute configuration of **10** preceded that of **9**, since a larger amount of **10** than of **9** was available and **10** would possess the same absolute configuration as **9**. The absolute configurations at C-1, C-2 and C-3 of **10** could be determined by conversion of its C-9a–C-1–C-2–C-3–C-3a moiety to arabitol. We attempted degradation of **10** to arabitol by oxidative ozonolysis, but this was unsuccessful. The C-1–C-9a and C-3–C-3a bonds of **10** seemed to be cleaved with fission of double bonds due to the presence of electron-donating groups (–OH) in the vicinity of double bonds.[12] Thus, **10** was derived to (−)-1,2-*trans*-2,3-*cis*-1,2,3-triacetoxy-2,3-dihydro-4-(4′-methoxyphenyl)phenalene (**15**) to protect the C-1–C-9a and C-3–C-3a bonds from ozonolysis (Scheme 1).[13] Compound **15** successfully gave the dimethyl ester of tri-*O*-acetyl-L-arabinaric acid (**16**) by oxidative ozonolysis and subsequent methylation. Compound **16** was then reduced and acetylated, giving arabitol pentaacetate (**17**) consisting of C-9a, C-1, C-2, C-3 and C-3a of **10**. The $[\alpha]_D^{25}$ value of **17**=−34° (*c* 0.07, $CHCl_3$), was close to that of authentic L-arabitol pentaacetate, −31° (*c* 5.20, $CHCl_3$), prepared from L-arabitol. This result identified the absolute configurations at the C-1, C-2 and C-3 of **10** as *S*, *R* and *S*, respectively, indicating that **10** was (1*S*,2*R*,3*S*)-(−)-2,3-dihydro-1,2,3-trihydroxy-4-(4′-methoxyphenyl)phenalene. The specific optical rotation of 4′-*O*-methyl ether of **9** was the same as that of **10**, and thus the absolute configuration of **9** was elucidated as (1*S*,2*R*,3*S*).

The absolute configurations of compounds **11** and **12**, **13**, and **14** were determined by chemical conversion to **10**, **9** and **16**, respectively (Scheme 1). Methylation of **11** gave **12**, and reduction of **12** afforded **10** and its 1-epimer, indicating that the absolute configuration at C-1 and C-2 of **11** and **12** was (2*S*,3*S*). Thus, compounds **11** and **12** were identified as (2*S*,3*S*)-(+)-2,3-dihydro-2,3-dihydroxy-4-(4′-hydroxyphenyl)-phenalen-1-one and (2*S*,3*S*)-(+)-2,3-dihydro-2,3-dihydroxy-4-(4′-methoxyphenyl)phenalen-1-one, respectively. Reduction of **13** gave **9** and its 3-epimer, revealing that **13** was (2*R*,3*S*)-(+)-2,3-dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)phenalen-1-one. Compound **14** was transformed to (−)-1,2-*trans*-2,3-*cis*-2,3-dihydro-1,2,3-trihydroxy-4-phenylphenalene (**18**) and its 3-epimer by reduction. Acetylation of **18** gave (−)-1,2-*trans*-2,3-*cis*-1,2,3-triacetoxy-2,3-dihydro-4-phenylphenalene (**19**), which was degraded to give **16**. These results identified the absolute configuration of the 1,2,3-trihydroxypropane moiety in **18** as (1*S*,2*R*,3*S*), indicating that **14** was (2*R*,3*S*)-(+)-2,3-dihydro-2,3-dihydroxy-9-phenylphenalen-1-one. The absolute configurations of **5** and **8** were not elucidated due to the small amounts available for analysis. However, the $[\alpha]_D^{25}$ value of **5**=+31° (*c* 0.03, MeOH), was similar to those of **11** and **12**, suggesting that the absolute configuration of **5** was (2*S*,3*S*).

Compounds **4**–**9** were not detected in extracts of unripe intact fruit peel on HPLC (data not shown), indicating that they were induced by wounding and subsequent inoculation, similarly to other phenylphenalenone derivatives isolated from banana fruit. No phenylphenalenones other than **1**–**3** and 2-phenyl-1,8-naphthalic anhydride (**20**) showed antifungal activities at less than 10 μg on TLC assays.[4,5] α-Hydroxyenone and 4-hydroxyphenyl moieties would be essential for the activities of 4- and 9-phenylphenalenones, and a naphthalic anhydride moiety would be needed for those of 2-phenyl-1,8-naphthalic anhydrides. The differences in the moieties essential for the activity between the phenylphenalenones and 2-phenyl-1,8-naphthalic anhydrides may be due to the differences in their antifungal mechanisms of action. In fact, 1,8-naphthalic anhydride showed antifungal activity at 0.1 μg on a TLC plate, suggesting that the lability of the anhydride group is involved in the antifungal mechanism of **1** and **20**.

Compounds **1**–**3** and **20** inhibited the spore germination of *C. musae* by 50% at 3.5, 3.2, 2.9 and 3.1 ppm, respectively, in aqueous solution. These strong activities suggested that these compounds could be candidates for lead compounds of fungicides.

Absolute configurations of **9**–**14** suggested that these compounds would be precursors or metabolites of each other in the biosynthetic pathway of phenylphenalenones. It has remained unknown how phenylphenalenones are biosynthesized from or to **3**, although biosynthetic precursors of **3** were shown to be two molecules of cinnamic acid and one molecule of malonate.[6] Luis et al. proposed a putative pathway in which both 4- and 9-phenylphenalenones are biosynthesized from a common intermediate, **9**.[7] However, the accumulation of **3** in wounded or inoculated unripe fruit did not accompany that of 4-phenylphenalenones such as **2**, and preceded that of other 4- and 9-phenylphenalenones in unripe fruit wounded followed by inoculation.[6] These results led us to notice an alternative biosynthetic pathway of phenylphenalenones, in which 4-phenylphenalenones are biosynthesized from 9-phenylphenalenones via **9**.[6] Here, we discussed the alternative pathway of phenylphenalenones including methyl ether derivatives in detail based on the results reported so far (Scheme 2). Compound **3** could give **21**[10] by oxidation at C-3, while **3** would also give **1** by oxidative decarboxylation as shown in our feeding experiment,[6] and **22**[4] by 2-*O*-methylation. Compound **21** may be converted to **13** by saturation at the C-2 double bond, probably giving **8** as a by-product. Compound **23**[9] could give **24**, **20** and **25**,[9] and also **14** and **26**[9] from **24**, respectively, similarly to **3**. The natural occurrence of phenalenones possessing a phenyl group at C-4 in banana fruit has not been found,[3–5,7–10] suggesting that **14** could not be reduced to **18**, while **13** is converted to **9**. Compound **9** is probably oxidized to **11** and then transformed to **2** by dehydration, since accumulation of **11** was observed prior to that of **2** in wounded and inoculated unripe fruit.[6] Compound **2** might be metabolized to **1** at a rate much lower than **3** to **1**.[6] The 9-substituents of 9-substituted phenalenones isolated from banana fruit are phenyl or 4′-hydroxyphenyl groups, while the 4-substituents of 4-substituted phenalenones are 4′-hydroxyphenyl or 4′-methoxyphenyl groups.[3–5,7–10] This suggests that first methylation of the 4′-hydroxyl group occurs at **9** to give **10**, and **10** might be transformed to **12**, **27**[8] and **28**,[5] the same as **9** to **1**. This pathway is not as simple as the Luis' pathway, but agrees with the results of the changes in the phenylphenalenone contents, the natural occurrence and the feeding experiments.[6] According to the pathway, **3** would be a major phytoalexin for banana fruit, and **1** and **2** seem to be minor phytoalexins which are formed by wounding followed by inoculation.[6] Structural diversity of phytoalexins might be one of the strategies by which plants protect themselves from severe damage.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal standard using Bruker AC300 and ARX500 spectrometers. Mass spectra were obtained with a Jeol JMS-600H mass spectrometer. UV spectra were determined with a Shimadzu UV-2200AI spectrometer. Optical rotation was recorded with a Jasco DIP-1000 polarimeter. Column chromatography was carried out on Wakogel C-200 (Wako Pure Chemical Industries) and ODS-AM 120-S50 (YMC). HPLC was performed with an ODS column (YMC, AQ-311, 6×100 mm) at a flow rate 1.0 mL/min with detection at UV 254 nm.

3.2. Plant materials and fungi

M. acuminata [AAA] cv. Buñgulan cultivated on Negulos Island, the Philippines, was used for the experiments. Unripe fruits, which were not treated with fungicides, were imported by Alter Trade Japan, Inc., Tokyo, Japan. *C. musae* (Berk. and Curt.) Arx. strains no. 1679 and 5501 were obtained from the Department of Scientific and Industrial Research, Mount Albert Research Centre, Auckland, New Zealand, and maintained on potato–sucrose–agar medium in the dark at 23°C. Suspensions of conidia were prepared from 5- to 9-day-old cultures. 10 mL of sterile water was poured onto the surface of the media, which was then rubbed with a glass bar. The suspension was filtered with paper (Crecia Corp., Wipers S-200) and diluted to 6.0×10^6 conidia/mL with sterile water.

3.3. Treatment and incubation

Unripe fruit were washed with water and wiped with 70% EtOH prior to experiments. Fruit were then wounded by rubbing with sandpaper (G-60) and soaked in a suspension of conidia of *C. musae* strain no. 1679 for 10 sec. Treated fruit were placed in plastic boxes (width 24 cm×depth 31 cm×height 10 cm) and kept in the dark at 17°C for 4–9 days. Wet cotton was placed in the boxes to maintain high humidity.

3.4. Isolation of compounds 4, 6, 7 and 9

The fruit peel (2.5 kg) was cut into 1×1 cm pieces after incubation, and extracted with 3.6 L of EtOAc for 3 days at room temperature. The EtOAc layer was concentrated to 500 mL, washed with H_2O (150 mL×3), dried over Na_2SO_4, and evaporated to dryness, yielding crude extract (5.2 g). The extract was subjected to silica gel column chromatography (800 g) using mixtures of toluene–EtOAc as the eluent to give the materials eluted with 50 and 100% EtOAc. The 50% EtOAc eluate (0.2 g) was subjected to silica gel column chromatography (25 g) using mixtures of *n*-hexane–EtOAc–MeOH (18:2:1, 16:4:1, 10:10:1 and 0:2:1), each 80 mL, as the eluent. The eluate was collected in fractions of 15 mL to give fractions no. 12–14. The materials from fraction no. 12 were subjected to silica gel column chromatography (20 g) using mixtures of *n*-hexane–EtOAc–MeOH (14:6:1, 12:8:1, and 10:10:1), each 80, 40 and 50 mL, as the eluent. The eluate was collected in fractions of 5 mL, and fractions no. 24 and 25 were purified by preparative HPLC with a linear gradient, MeOH–H_2O (2:3 to 11:9), over 20 min. The materials eluted at t_R 19.2 and 20.8 min were collected to give **7** (0.2 mg) as a white powder and **4** (0.2 mg) as an orange powder, respectively. The materials from fractions no. 13

and 14 obtained from the second silica gel column chromatography were subjected to silica gel column chromatography (5 g) using mixtures of *n*-hexane–EtOAc–MeOH (14:6:1 and 12:8:1), each 20 mL, as the eluent. The eluate was collected in fractions of 3 mL, and fraction no. 9 was purified by preparative HPLC with elution with MeOH–H_2O (23:27). Collection of the material eluted at t_R 27.6 min yielded **6** (0.3 mg) as a white powder. The 100% EtOAc eluate (0.2 g) was subjected to silica gel column chromatography (15 g) using mixtures of *n*-hexane–EtOAc–MeOH (14:6:1, 12:8:1, 10:10:1 and 0:20:1), each 70 mL, as the eluent. The eluate was collected in fractions of 30 mL, and the materials from fractions no. 7 and 8 were purified by preparative HPLC with elution with MeOH–H_2O (49:51), and collection of the material eluted at t_R 15.0 min yielded **9** (0.3 mg) as white powder.

3.5. Isolation of compounds 5, 8 and 10–14

The fruit peel (3.3 kg) was extracted with 4 L of EtOAc, yielding crude extract (6.0 g) in the manner described above. The extract was subjected to silica gel column chromatography (250 g) using mixtures of toluene–EtOAc (9:1, 4:1, 1:1 and 0:1), each 750 mL, as the eluent. The eluate was collected in fractions of 250 mL to give fractions no. 6, 7, 9–11. First, the materials from fractions no. 6 and 7 were subjected to silica gel column chromatography using (25 g) mixtures of *n*-hexane–$CHCl_3$–MeOH (10:10:1) as the eluent. The eluate was collected in fractions of 10 mL, and fractions no. 8–10 were then subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 7–10 were purified by preparative HPLC with CH_3CN–H_2O (2:3) as the eluent. The material eluted at t_R 14.9 min was collected to give **12** (30.4 mg). The materials from fraction no. 8 were subjected to silica gel column chromatography (25 g) using mixtures of *n*-hexane–$CHCl_3$–MeOH (8:12:1) as the eluent. The eluate was collected in fractions of 10 mL, and fractions no. 5–8 were then subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 5 mL, and fractions no. 13–30 were purified by preparative HPLC with CH_3CN–H_2O (2:3) as the eluent. The material eluted at t_R 12.8 min was collected to give **14** (8.0 mg) as a white powder. Second, the materials from fraction no. 9 were subjected to silica gel column chromatography (25 g) using mixtures of *n*-hexane–$CHCl_3$–MeOH (8:12:1) as the eluent. The eluate was collected in fractions of 10 mL, and fractions no. 17–21 were then subjected to ODS column chromatography (17 g) using a mixture of CH_3CN–H_2O (7:13) as the eluent. The eluate was collected in fractions of 10 mL, and fractions no. 9 and 10 were purified by preparative HPLC with CH_3CN–H_2O (8:17) as the eluent. The material eluted at t_R 11.4 min was collected to give **11** (11.5 mg) as a white powder. Third, the materials from fraction no. 10 were subjected to silica gel column chromatography (25 g) using a mixture of *n*-hexane–$CHCl_3$–MeOH (5:15:1) as the eluent. The eluate was collected in fractions of 50 mL, and fraction no. 4 was then subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 8 mL, and fractions no. 7 and 8 were purified by preparative HPLC with CH_3CN–H_2O (1:3) as the eluent. The material eluted at t_R 16.4 min was collected to give **5** (0.3 mg) as a white powder. Fraction no. 9 was purified by preparative HPLC with CH_3CN–H_2O (1:3) as the eluent. The material eluted at t_R 21.6 min was collected to give **8** (0.6 mg) as a white powder. Fractions no. 10–12 were again purified by ODS column chromatography (17 g) using a mixture of CH_3CN–H_2O (3:7) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 9 and 10 were concentrated to give **13** (6.7 mg). Fourth, the materials from fraction no. 11 were subjected to silica gel column chromatography (25 g) using a mixture of *n*-hexane–$CHCl_3$–MeOH (11:9:1) as the eluent. The eluate was collected in fractions of 30 mL, and fraction no. 4 was then subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 10 and 11 were purified by preparative HPLC with CH_3CN–H_2O (7:13) as the eluent. The material eluted at t_R 12.6 min was collected to give **10** (3.1 mg) as colorless needles.

3.5.1. 4-(3′,4′-Dihydroxyphenyl)-2-hydroxyphenalen-1-one (4). UV λ_{max} (MeOH) nm (ε): 243 (13,000), 283 (11,000), 328 (4000), 338 (4000), 440 (4300); EIMS (probe) 70 eV *m/z* (%): 304 $[M]^+$ (100), 303 $[M-H]^+$ (16), 287 (49), 286 (50); HREIMS *m/z* (M^+): calculated for $C_{19}H_{12}O_4$, 304.0735; found 304.0731; NMR δ_H (300 MHz, acetone-d_6): 6.88 (1H, dd, *J*=8.1, 2.1 Hz, 6′-*H*), 7.02 (1H, d, *J*=2.1 Hz, 2′-*H*), 7.03 (1H, d, *J*=8.1 Hz, 5′-*H*), 7.29 (1H, s, 3-*H*), 7.62 (1H, d, *J*=8.5 Hz, 5-*H*), 7.89 (1H, dd, *J*=8.0, 7.4 Hz, 8-*H*), 8.08 (1H, d, *J*=8.5 Hz, 6-*H*), 8.09 (1H, s, 2,3′ or 4′-O*H*), 8.25 (1H, s, 2,3′or 4′-O*H*), 8.26 (1H, s, 2,3′ or 4′-O*H*), 8.43 (1H, dd, *J*=8.0, 1.2 Hz, 7-*H*), 8.69 (1H, dd, *J*=7.4, 1.2 Hz, 9-*H*).

3.5.2. (+)-2,3-*cis*-4-(3′,4′-Dihydroxyphenyl)-2,3-dihydro-2,3-dihydroxyphenalen-1-one (5). UV λ_{max} (MeOH) nm (ε): 268 (23,000), 282 (13,000); EIMS (probe) 70 eV *m/z* (%): 304 $[M-H_2O]^+$ (100), 303 $[M-H_2O-H]^+$ (15), 287 (47), 286 (44), 258 (20); HREIMS *m/z* ($M-H_2O^+$): calculated for $C_{19}H_{12}O_4$, 304.0735; found 304.0724; NMR δ_H (500 MHz, acetone-d_6): 4.79 (1H, d, *J*=3.3 Hz, 2-*H*), 5.34 (1H, d, *J*=3.3 Hz, 3-*H*), 6.94 (1H, d, *J*=8.0 Hz, 5′-*H*), 7.01 (1H, dd, *J*=8.0, 2.0 Hz, 6′-*H*), 7.18 (1H, d, *J*=2.0 Hz, 2′-*H*), 7.61 (1H, d, *J*=8.5 Hz, 5-*H*), 7.68 (1H, dd, *J*=8.1, 7.0 Hz, 8-*H*), 8.04 (1H, d, *J*=8.5 Hz, 6-*H*), 8.13 (1H, dd, *J*=7.0, 1.1 Hz, 9-*H*), 8.69 (1H, dd, *J*=8.1, 1.1 Hz, 7-*H*).

3.5.3. 2-(4′-Hydroxyphenyl)naphthal-8-formyl-1-carboxylic anhydride (6). UV λ_{max} (MeOH) nm (ε): 227 (19,000), 245 (14,000), 290 (5300), 303 (4700), 332 (4100); EIMS (probe) 70 eV *m/z* (%): 292 $[M]^+$ (100), 275 $[M-OH]^+$ (11), 264 (63), 247 (68); HREIMS *m/z* (M^+): calculated for $C_{18}H_{12}O_4$, 292.0735; found 292.0742; NMR δ_H (300 MHz, acetone-d_6): 6.89 (1H, s, 9-*H*), 6.89 (2H, d, *J*=8.6 Hz, 3′,5′-*H*), 7.02 (1H, brs, 9-O*H*), 7.30 (2H, d, *J*=8.6 Hz, 2′,6′-*H*), 7.59 (1H, d, *J*=8.5 Hz, 3-*H*), 7.67 (1H, dd, *J*=8.2, 6.5 Hz, 6-*H*), 7.78 (1H, dd, *J*=6.5, 1.0 Hz, 5-*H*), 8.07 (1H, dd, *J*=8.2, 1.0 Hz, 7-*H*), 8.24 (1H, d, *J*=8.5 Hz, 4-*H*), 8.47 (1H, brs, 4′-O*H*).

3.5.4. 7-(4′-Hydroxyphenyl)naphthal-8-formyl-1-carboxylic anhydride (7). UV λ_{max} (MeOH) nm (ε): 228

(17,000), 245 (11,000), 274 (6700), 308 (3300), 319 (3400), 340 (2800); EIMS (probe) 70 eV *m/z* (%): 292 $[M]^+$ (100), 275 $[M-OH]^+$ (37), 264 (58), 247 (63); HREIMS *m/z* (M^+): calculated for $C_{18}H_{12}O_4$, 292.0735; found 292.0732; NMR δ_H (acetone-d_6, 300 MHz): 6.67 (1H, s, 9-*H*), 6.99 (2H, d, *J*=8.7 Hz, 3′,5′-*H*), 7.05 (1H, brs, 1-O*H*), 7.49 (2H, d, *J*=8.7 Hz, 2′,6′-*H*), 7.61 (1H, d, *J*=8.5 Hz, 6-*H*), 7.76 (1H, dd, *J*=8.3, 7.2 Hz, 3-*H*), 8.12 (1H, d, *J*=8.5 Hz, 5-*H*), 8.33 (1H, dd, *J*=8.3, 1.1 Hz, 4-*H*), 8.40 (1H, dd, *J*=7.2, 1.1 Hz, 2-*H*), 8.63 (1H, brs, 4′-O*H*).

3.5.5. (−)-2,3-*cis*-2,3-Dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)phenalen-1-one (8). $[\alpha]_D^{25}=-106°$ (*c* 0.06, MeOH); UV λ_{max} (MeOH) nm (ε): 246 (23,000), 287 (9800), 320 (6100); EIMS (probe) 70 eV *m/z* (%): 306 $[M]^+$ (6), 288 $[M-H_2O]^+$ (59), 287 $[M-H_2O-H]^+$ (100), 271 (20); HREIMS *m/z* (M^+): calculated for $C_{19}H_{14}O_4$, 306.0892; found 306.0869; NMR δ_H (500 MHz, acetone-d_6): 4.28 (1H, d, *J*=4.0 Hz, 2-O*H*), 4.47 (1H, d, *J*=3.0 Hz, 3-O*H*), 4.91 (1H, dd, *J*=4.0, 3.7 Hz, 2-*H*), 5.24 (1H, dd, *J*=3.7, 3.0 Hz, 3-*H*), 6.87 (2H, d, *J*=8.6 Hz, 3′,5′-*H*), 7.20 (2H, d, *J*=8.6 Hz, 2′,6′-*H*), 7.51 (1H, d, *J*=8.4 Hz, 8-*H*), 7.63 (1H, dd, *J*=8.2, 7.0 Hz, 5-*H*), 7.74 (1H, d, *J*=7.0 Hz, 4-*H*), 8.03 (1H, d, *J*=8.2 Hz, 6-*H*), 8.18 (1H, d, *J*=8.4 Hz, 7-*H*), 8.38 (1H, s, 4′-O*H*).

3.5.6. (1*S*,2*R*,3*S*)-(−)-2,3-Dihydro-1,2,3-trihydroxy-4-(4′-hydroxyphenyl)phenalene (9). $[\alpha]_D^{25}=-5°$ (*c* 0.49, MeOH); UV λ_{max} (MeOH) nm (ε): 231 (24,000), 259 (15,000), 290 (6800); EIMS (probe) 70 eV *m/z* (%): 308 $[M]^+$ (46), 290 $[M-H_2O]^+$ (53), 273 (100), 272 (93); HREIMS *m/z* (M^+): calculated for $C_{19}H_{16}O_4$, 308.1048; found 308.1041; NMR δ_H (300 MHz, acetone-d_6): 2.46 (1H, s, 1-O*H*), 2.54 (1H, d, 3-O*H*), 2.82 (1H, d, 2-O*H*), 3.85 (1H, dd, *J*=9.7, 3.2 Hz, 2-*H*), 5.02 (1H, d, *J*=3.2 Hz, 3-*H*), 5.28 (1H, d, *J*=9.7 Hz, 1-*H*), 6.94 (2H, d, *J*=8.7 Hz, 3′,5′-*H*), 7.47 (1H, d, *J*=8.1 Hz, 5-*H*), 7.49 (2H, d, *J*=8.7 Hz, 2′,6′-*H*), 7.58 (1H, dd, *J*=8.5, 7.6 Hz, 8-*H*), 7.84 (1H, d, *J*=8.1 Hz, 6-*H*), 7.84 (1H, d, *J*=7.6 Hz, 7-*H*), 7.90 (1H, d, *J*=8.5 Hz, 9-*H*); NMR δ_C (125 MHz, acetone-d_6): 70.0 (C-3), 70.5 (C-1), 76.1 (C-2), 116.1 (C-3′,5′), 124.3 (C-9), 126.8 (C-8), 127.8 (C-7), 129.3 (C-6), 129.5 (C-9b), 129.9 (C-5), 132.4 (C-2′,6′), 132.4 (C-3a), 133.4 (C-1′), 133.7 (C-6a), 139.3 (C-9a), 141.4 (C-4), 158.1 (C-4′). Ethereal diazomethane was added to **9** (4.9 mg) dissolved in 2 mL of MeOH, and the solution was left at room temperature for 2 h. The solvent was evaporated, and the residue was subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 8–10 were evaporated to give **10** (3.9 mg). $[\alpha]_D^{25}=-34°$ (*c* 0.39, $CHCl_3$); the ^1H NMR spectrum was identical to that of natural **10**.

3.5.7. (1*S*,2*R*,3*S*)-(−)-2,3-Dihydro-1,2,3-trihydroxy-4-(4′-methoxyphenyl)phenalene (10). Mp 92–96°C; $[\alpha]_D^{25}=-35°$ (*c* 0.31, $CHCl_3$). See the literature[7] for the other spectral data.

3.5.8. (2*S*,3*S*)-(+)-2,3-Dihydro-2,3-dihydroxy-4-(4′-hydroxyphenyl)phenalen-1-one (11). $[\alpha]_D^{25}=+56°$ (*c* 1.15, MeOH); NMR δ_H (500 MHz, acetone-d_6): 4.50 (1H, brs, 2-O*H*), 4.58 (1H, brs, 3-O*H*), 4.79 (1H, d, *J*=3.1 Hz, 2-*H*), 5.28 (1H, d, *J*=3.1 Hz, 3-*H*), 6.98 (2H, d, *J*=8.6 Hz, 3′,5′-*H*), 7.54 (2H, d, *J*=8.6 Hz, 2′,6′-*H*), 7.61 (1H, d, *J*=8.5 Hz, 5-*H*), 147.67 (1H, dd, *J*=8.1, 7.1 Hz, 8-*H*), 8.05 (1H, d, *J*=8.5 Hz, 6-*H*), 8.13 (1H, d, *J*=7.1 Hz, 9-*H*), 8.26 (1H, d, *J*=8.1 Hz, 7-*H*), 8.55 (1H, brs, 4′-O*H*); NMR δ_C (125 MHz, acetone-d_6): 71.8 (C-3), 78.2 (C-2), 116.4 (C-3′,5′), 126.6 (C-9), 126.9 (C-8), 129.5 (C-6), 130.1 (C-9a), 131.0 (C-5), 131.4 (C-3a), 132.1 (C-2′,6′), 132.2 (C-9b), 132.5 (C-1′), 134.0 (C-6a), 135.4 (C-7), 142.7 (C-4), 158.5 (C-4′), 199.5 (C-1). See the literature[4] for the other spectral data; the assignment of signals in the ^1H NMR spectrum reported in the literature were partially in error. Ethereal diazomethane was added to **11** (5.0 mg) dissolved in 2 mL of MeOH, and the solution was left at room temperature for 2 h. The solvent was evaporated, and the residue was subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 9 and 10 were evaporated to give **12** (1.7 mg). $[\alpha]_D^{25}=+65°$ (*c* 0.17, $CHCl_3$); the ^1H NMR spectrum was identical to that of natural **12**.

3.5.9. (2*S*,3*S*)-(+)-2,3-Dihydro-2,3-dihydroxy-4-(4′-methoxyphenyl)phenalen-1-one (12). $[\alpha]_D^{25}=+65°$ (*c* 0.70, $CHCl_3$); NMR δ_H (500 MHz, $CDCl_3$): 2.96 (1H, s, 3-O*H*), 3.88 (3H, s, 4′-OC*H*$_3$), 4.23 (1H, s, 2-O*H*), 4.72 (1H, d, *J*=3.1 Hz, 2-*H*), 5.30 (1H, d, *J*=3.1 Hz, 3-*H*), 7.02 (2H, d, *J*=8.7 Hz, 3′,5′-*H*), 7.57 (1H, d, *J*=8.6 Hz, 5-*H*), 7.60 (2H, d, *J*=8.7 Hz, 2′,6′-*H*), 7.63 (1H, dd, *J*=8.0, 7.1 Hz, 8-*H*), 7.96 (1H, d, *J*=8.6 Hz, 6-*H*), 8.15 (1H, d, *J*=8.0 Hz, 7-*H*), 8.23 (1H, d, *J*=7.1 Hz, 9-*H*); NMR δ_C (125 MHz, $CDCl_3$): 55.4 (O*C*H$_3$), 69.9 (C-3), 76.2 (C-2), 113.9 (C-3′,5′), 125.8 (C-8), 126.6 (C-9), 127.4 (C-6a), 127.9 (C-3a), 128.9 (C-6), 129.9 (C-5), 130.7 (C-2′,6′), 131.0 (C-9b), 132.0 (C-1′), 132.7 (C-9a), 135.0 (C-7), 142.6 (C-4), 159.5 (C-4′), 198.2 (C-1). See the literature[4,9] for the other spectral data; the assignment of signals in the ^1H NMR spectrum reported in the literature were partially in error. Compound **12** (39 mg) in 12 mL of EtOH was stirred at room temperature for 2 h after addition of $NaBH_4$ (20 mg). The solution was acidified to pH 3 with 1N HCl, and then extracted with EtOAc (15 mL×3). The organic layer was washed with H_2O (10 mL×3), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fractions no. 9–12 and 14–17 were evaporated to give **10** (23.1 mg) and 1-epimer of **10** (8.4 mg), respectively. Compound **10**. $[\alpha]_D^{25}=-35°$ (*c* 2.31, $CHCl_3$); the ^1H NMR spectrum was identical to that of natural **10**. 1-Epimer of **10**. $[\alpha]_D^{25}=-121°$ (*c* 0.84, $CHCl_3$); NMR δ_H (500 MHz, $CDCl_3$): 3.50–3.70 (3H, brs, 1,2, and 3-O*H*), 4.08 (1H, dd, *J*=2.1, 2.1 Hz, 2-*H*), 5.05 (1H, d, *J*=2.1 Hz, 1 or 3-*H*), 5.08 (1H, d, *J*=2.1 Hz, 1 or 3-*H*), 6.97 (2H, d, *J*=8.7 Hz, 3′,5′-*H*), 7.47 (1H, d, *J*=8.4 Hz, 5-*H*), 7.48 (2H, d, *J*=8.7 Hz, 2′,6′-*H*), 7.52 (1H, dd, *J*=8.0, 6.7 Hz, 8-*H*), 7.60 (1H, d, *J*=6.7 Hz, 7-*H*), 7.88 (1H, d, *J*=8.0 Hz, 9-*H*), 7.88 (1H, d, *J*=8.4 Hz, 6-*H*).

3.5.10. (2*R*,3*S*)-(−)-2,3-Dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)phenalen-1-one (13). $[\alpha]_D^{25}=-137°$ (*c* 0.56, MeOH). See the literature[10] for the other spectral data. Compound **13** (5.5 mg) in 2 mL of EtOH was stirred at room temperature for 2 h after addition of $NaBH_4$ (2 mg).

The solution was acidified to pH 3 with 1N HCl, and then extracted with EtOAc (5 mL×3). The organic layer was washed with H_2O (3 mL×3), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (11:9) as the eluent. The eluate was collected in fractions of 15 mL. Fractions no. 4–7 were evaporated and then purified by preparative HPLC with MeOH–H_2O (1:1) as the eluent. The materials eluted at t_R 11.6 min and t_R 14.4 min were collected to give **9** (0.6 mg) and 3-epimer of **9** (3.7 mg), respectively. Compound **9**. $[\alpha]_D^{25}=-6°$ (*c* 0.06, MeOH); the ^1H NMR spectrum was identical to that of natural **9**. 3-Epimer of **9**. $[\alpha]_D^{25}=+57°$ (*c* 0.37, MeOH); NMR δ_H (500 MHz, acetone-d_6): 4.44 (1H, dd, *J*=2.5, 1.8 Hz, 2-*H*), 4.95 (1H, d, *J*=1.8 Hz, 1 or 3-*H*), 4.98 (1H, d, *J*=2.5 Hz, 1 or 3-*H*), 6.93 (2H, d, *J*=8.7 Hz, 3′,5′-*H*), 7.46 (1H, d, *J*=8.5 Hz, 5-*H*), 7.50 (1H, dd, *J*=8.2, 7.0 Hz, 8-*H*), 7.51 (2H, d, *J*=8.7 Hz, 2′,6′-*H*), 7.57 (1H, d, *J*= 7.0 Hz, 7-*H*), 7.87 (1H, d, *J*=8.2 Hz, 9-*H*), 7.89 (1H, d, *J*=8.5 Hz, 6-*H*), 8.39 (1H, brs, 4′-O*H*).

3.5.11. (2*R*,3*S*)-(−)-2,3-Dihydro-2,3-dihydroxy-9-phenylphenalen-1-one (14). $[\alpha]_D^{25}=-101°$ (*c* 0.80, $CHCl_3$); NMR δ_C ($CDCl_3$, 125 MHz): 74.4 (C-3), 79.3 (C-2), 125.0 (C-6a), 125.0 (C-4), 127.0 (C-5), 127.6 (C-4′), 127.9 (C-6), 128.0 (C-3′,5′), 128.7 (C-2′,6′), 129.8 (C-8), 130.0 (C-3a), 132.4 (C-9a), 133.6 (C-7), 133.8 (C-9b), 140.8 (C-1′), 142.8 (C-9), 197.8 (C-1). See the literature[11] for the other spectral data.

3.5.12. (1*S*,2*R*,3*S*)-(+)-1,2,3-Triacetoxy-2,3-dihydro-4-(4′-methoxyphenyl)phenalene (15). Compound **10** (15.5 mg) was dissolved in pyridine (0.5 mL), and stirred at room temperature for 48 h after addition of acetic anhydride (0.3 mL). The mixture was fractionated between EtOAc and H_2O, and the organic layer (20 mL) was washed with H_2O (10 mL×3), dried over Na_2SO_4, filtered and concentrated to dryness, giving **15** (20.6 mg) as a yellow oil. $[\alpha]_D^{25}=+141°$ (*c* 0.42, $CHCl_3$); UV λ_{max} (MeOH) nm (ε): 230 (66,000), 243 (18,000), 287 (11,000), 302 (7600); EIMS (probe) 70 eV *m/z* (%): 448 $[M]^+$ (21), 345 (18), 329 (35), 303 (18), 287 (100); HREIMS *m/z* (M^+): calculated for $C_{26}H_{24}O_7$, 448.1522; found 448.1520; NMR δ_H ($CDCl_3$, 500 MHz): 1.96 (3H, s, 3-OCOC*H*$_3$), 2.00 (3H, s, 1-OCOC*H*$_3$), 2.25 (3H, s, 2-OCOC*H*$_3$), 3.85 (3H, s, 4′-OC*H*$_3$), 5.42 (1H, dd, *J*=10.2, 3.0 Hz, 2-*H*), 6.57 (1H, d, *J*=3.0 Hz, 3-*H*), 6.75 (1H, d, *J*=10.2 Hz, 1-*H*), 6.96 (2H, d, *J*=8.5 Hz, 3′,5′-*H*), 7.20 (2H, d, *J*=8.5 Hz, 2′,6′-*H*), 7.41 (1H, d, *J*=7.2 Hz, 9-*H*), 7.44 (1H, d, *J*=8.4 Hz, 5-*H*), 7.54 (1H, dd, *J*=8.1, 7.2 Hz, 8-*H*), 7.87 (1H, d, *J*=8.1 Hz, 7-*H*), 7.91 (1H, d, *J*=8.4 Hz, 6-*H*); NMR δ_C ($CDCl_3$, 125 MHz): 20.8–21.1 (–OCO*C*H$_3$×3), 55.3 (O*C*H$_3$-4′), 67.7 (C-3), 69.1 (C-1), 72.3 (C-2), 113.8 (C-3′,5′), 123.9 (C-9), 125.8 (C-8), 126.2 (C-3a), 128.1 (C-7), 128.3 (C-9b), 129.3 (C-6), 129.3 (C-5), 130.3 (C-2′,6′), 131.6 (C-9a), 132.0 (C-1′), 132.4 (C-6a), 141.2 (C-4), 159.3 (C-4′), 169.2–171.0 (–O*C*OCH$_3$×3).

3.5.13. Dimethyl ester of tri-*O*-acetyl-L-arabinaric acid (16). Compound **15** (20.4 mg) was dissolved in AcOH (3 mL), and O_3 gas from an ozone generator (Japan Ozone Co. Ltd, Model O-3-2) was passed through the solution for 24 h at room temperature. After N_2 gas was passed through the solution, 0.5 mL of 30% hydrogen peroxide was then added, and the mixture was stirred overnight at room temperature and for 1.5 h at 100°C. The solution was concentrated, dissolved in MeOH (1 mL), and then ethereal diazomethane was added. The solvent was evaporated, and the residue was subjected to silica gel column chromatography (3 g) using a mixture of *n*-hexane–EtOAc (3:1) as the eluent. The eluate was collected in fractions of 5 mL, and fractions no. 5 and 6 were purified by preparative HPLC with MeOH–H_2O (2:3) as the eluent. The material eluted at t_R 11.2 min was collected to give **16** as a colorless oil (0.5 mg). $[\alpha]_D^{25}=-31°$ (*c* 0.05, $CHCl_3$); EIMS (probe) 70 eV *m/z* (%): 303 $[M-OCH_3]^+$ (6), 275 $[M-CO_2CH_3]^+$ (30), 261 (33), 203 (50), 173 (100); HREIMS *m/z* ($M-CO_2CH_3^+$): calculated for $C_{11}H_{15}O_8$, 275.0767; found 275.0764; NMR δ_H (500 MHz, $CDCl_3$): 2.08 (3H, s, OCOC*H*$_3$), 2.13 (3H, s, OCOC*H*$_3$), 2.19 (3H, s, OCOC*H*$_3$), 3.74 (3H, s, COOC*H*$_3$), 3.75 (3H, s, COOC*H*$_3$), 5.13 (1H, d, *J*=8.6 Hz, 4-*H*), 5.48 (1H, d, *J*= 2.8 Hz, 2-*H*), 5.77 (1H, dd, *J*=8.6, 2.8 Hz, 3-*H*); NMR δ_C (125 MHz, $CDCl_3$): 20.3–20.5 (CO*C*H$_3$×3), 53.1 (CO_2CH_3), 53.2 (CO_2CH_3), 68.9 (C-3), 69.4 (C-2 or 4), 69.6 (C-2 or 4), 167.0 (*C*O$_2$CH$_3$), 167.1 (*C*O$_2$CH$_3$), 169.3–169.8 (*C*OCH$_3$×3).

3.5.14. L-Arabitol pentaacetate (17). $NaBH_4$ (14 mg) was added to MeOH (0.5 mL) solution of **16** (2.2 mg), and the mixture was stirred for 2 h at 100°C. H_2O was added to the solution cooled to room temperature, which was then acidified to pH 3 with 1N HCl, and concentrated to dryness. The residual white powder was partly dissolved in EtOH (5 mL), and the EtOH layer was concentrated to dryness, dissolved in pyridine (1 mL), and stirred at room temperature for 18 h after adding acetic anhydride (0.5 mL). The mixture was fractionated between EtOAc and H_2O after stopping the reaction by adding H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was subjected to silica gel column chromatography (1.5 g) using a mixture of *n*-hexane–EtOAc (3:1) as the eluent. The eluate was collected in fractions of 2 mL, and fractions no. 6–10 were evaporated to give **17** (0.7 mg) as a colorless oil. $[\alpha]_D^{25}=-34°$ (*c* 0.07, $CHCl_3$); EIMS (probe) 70 eV *m/z* (%): 289 $[M-CH_2OAc]^+$ (32), 217 $[M-C_2H_3(OAc)_2]^+$ (64), 187 (77), 145 $[M-C_3H_4(OAc)_3]^+$ (89), 115 (100); HREIMS *m/z* ($M-CH_2OAc^+$): calculated for $C_{12}H_{17}O_8$, 289.0923; found 289.0923; NMR δ_H (500 MHz, $CDCl_3$): 2.04–2.13 (15H, s×5, COC*H*$_3$×5), 3.95 (1H, dd, *J*=11.7, 7.0 Hz, 1 or 5-*H*), 4.15 (1H, dd, *J*=12.5, 5.0 Hz, 1 or 5-*H*), 4.24 (1H, dd, *J*= 12.5, 2.7 Hz, 1 or 5-*H*), 4.28 (1H, dd, *J*=11.7, 5.0 Hz, 1 or 5-*H*), 5.15–5.42 (3H, m, 2-4-*H*); NMR δ_C (125 MHz, $CDCl_3$): 20.5–20.8 (CO*C*H$_3$×5), 61.8 (C-1 or 5), 62.0 (C-1 or 5), 68.1 (C-2), 68.1 (C-4), 68.4 (C-3), 169.6–170.6 (*C*OCH$_3$×5). L-Arabitol (20 mg; Aldrich) was dissolved in pyridine (2 mL), and stirred for 24 h at room temperature after adding acetic anhydride (1 mL). H_2O was added to the mixture, and followed by fractionation between EtOAc and H_2O. The organic layer (20 mL) was washed with H_2O (10 mL×3), dried over Na_2SO_4, filtered and concentrated to dryness, giving L-arabitol pentaacetate as a colorless oil (48 mg).

3.5.15. (1*R*,2*S*,3*R*)-(−)-2,3-Dihydro-1,2,3-trihydroxy-4-phenylphenalene (18). Compound **14** (27.5 mg) in 6 mL of EtOH was stirred at room temperature for 1.5 h after

addition of $NaBH_4$ (10 mg). The solution was acidified to pH 3 with 1N HCl, and then extracted with EtOAc (20 mL). The organic layer was washed with H_2O (10 mL×3), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was subjected to ODS column chromatography (17 g) using a mixture of MeOH–H_2O (3:2) as the eluent. The eluate was collected in fractions of 15 mL, and fraction no. 9 was evaporated to give **18** (6.0 mg) as colorless needles and 3-epimer of **18** (15.1 mg) as a white powder. Compound **18**. Mp 173–177°C; $[\alpha]_D^{25}=-4°$ (*c* 0.60, $CHCl_3$); UV λ_{max} (EtOH) nm (ε): 232 (48,000), 243 (50,000), 278 (9100), 290 (11,000), 302 (7300); EIMS (probe) 70 eV *m/z* (%): 292 $[M]^+$ (49), 274 $[M-H_2O]^+$ (42), 257 (93), 256 (100), 228 (27), 215 (32), 202 (24); HREIMS *m/z* (M^+): calculated for $C_{19}H_{16}O_3$, 292.1099; found 292.1105; NMR δ_H (500 MHz, $CDCl_3$): 2.79 (1H, brs, O*H*), 3.05 (1H, brs, O*H*), 3.33 (1H, brs, O*H*), 3.75 (1H, dd, *J*=9.7, 2.8 Hz, 2-*H*), 4.94 (1H, d, *J*=2.8 Hz, 3-*H*), 5.22 (1H, d, *J*=9.7 Hz, 1-*H*), 7.31–7.52 (5H, m, 2′,3′,4′,5′,6′-*H*), 7.44 (1H, d, *J*=8.4 Hz, 5-*H*), 7.51 (1H, dd, *J*=8.2, 7.3 Hz, 8-*H*), 7.56 (1H, d, *J*=7.3 Hz, 9-*H*), 7.79 (1H, d, *J*=8.2 Hz, 7-*H*), 7.87 (1H, d, *J*=8.4 Hz, 6-*H*); NMR δC (125 MHz, $CDCl_3$): 68.2 (C-3), 70.1 (C-2), 74.9 (C-2), 123.0 (C-7), 126.1 (C-8), 127.4 (C-4′), 127.5 (C-9), 127.5 (C-9b), 128.1 (C-2′,6′), 128.6 (C-5), 128.8 (C-6), 129.7 (C-3′,5′), 129.8 (C-3a), 132.7 (C-6a), 135.7 (C-9a), 140.4 (C-1′), 140.7 (C-4). 3-Epimer of **18**. $[\alpha]_D^{25}=+35°$ (*c* 0.75, $CHCl_3$); NMR δ_H (500 MHz, $CDCl_3$): 2.11 (1H, d, *J*=5.4 Hz, 1, 2, or 3-O*H*), 2.86 (1H, d, *J*=5.4 Hz, 1, 2, or 3-O*H*), 3.19 (1H, d, *J*=5.4 Hz, 1, 2, or 3-O*H*), 4.28 (1H, ddd, *J*=5.6, 5.4, 5.4 Hz, 2-*H*), 5.03 (1H, dd, *J*=5.6, 5.4 Hz, 1 or 3-*H*), 5.23 (1H, dd, *J*=5.4, 5.4 Hz, 1 or 3-*H*), 7.41–7.55 (5H, m, 2′-6′-*H*), 7.47 (1H, d, *J*=8.4 Hz, 5-*H*), 7.57 (1H, dd, *J*=8.4, 7.0 Hz, 8-*H*), 7.73 (1H, d, *J*=7.0 Hz, 6-*H*), 7.87 (1H, d, *J*=8.4 Hz, 7-*H*), 7.90 (1H, d, *J*=8.4 Hz, 9-*H*).

3.5.16. (1*R*,2*S*,3*R*)-(+)-1,2,3-Triacetoxy-2,3-dihydro-4-phenylphenalene (19). Compound **18** (5.9 mg) was dissolved in pyridine (0.5 mL), and stirred at room temperature for 48 h after addition of acetic anhydride (0.3 mL). The mixture was concentrated to dryness, giving **19** (8.5 mg) as a colorless oil. $[\alpha]_D^{25}=+172°$ (*c* 0.85, $CHCl_3$); UV λ_{max} (EtOH) nm (ε): 229 (61,000), 278 (8400), 288 (9400), 301 (6300); EIMS (probe) 70 eV *m/z* (%): 418 $[M]^+$ (5), 359 $[M-OAc]^+$ (4), 358 (14), 315 (25), 299 (23), 273 (23), 257 (100); HREIMS *m/z* (M^+): calculated for $C_{25}H_{22}O_6$, 418.1416; found 418.1419; NMR δ_H (500 MHz, $CDCl_3$): 1.93 (3H, s, 3-OCOC*H*$_3$), 1.98 (3H, s, 2-OCOC*H*$_3$), 2.25 (3H, s, 1-OCOC*H*$_3$), 5.44 (1H, dd, *J*=10.1, 3.1 Hz, 2-*H*), 6.53 (1H, d, *J*=3.1 Hz, 3-*H*), 6.72 (1H, d, *J*=10.1 Hz, 1-*H*), 7.24–7.45 (5H, m, 2′,3′,4′,5′,6′-*H*), 7.42 (1H, d, *J*=7.3 Hz, 9-*H*), 7.47 (1H, d, *J*=8.4 Hz, 5-*H*), 7.56 (1H, dd, *J*=8.2, 7.3 Hz, 8-*H*), 7.88 (1H, d, *J*=8.2 Hz, 7-*H*), 7.94 (1H, d, *J*=8.4 Hz, 6-*H*); NMR δ_C (125 MHz, $CDCl_3$): 20.7–21.1 (–OCO*C*H$_3$×3), 67.4 (C-3), 69.1 (C-1), 72.1 (C-2), 124.0 (C-9b), 126.0 (C-8), 126.2 (C-3a), 127.8 (C-7), 128.2 (C-9), 128.2 (C-4′), 128.3 (C-2′,6′), 128.8 (C-6), 129.1 (C-3′,5′), 129.3 (C-5), 131.7 (C-9a), 132.6 (C-6a), 139.8 (C-1′), 141.5 (C-4), 169.1–171.0 (–O*C*OCH$_3$×3). Ozonolysis of **19** (8.5 mg) gave **16** (0.3 mg) in the same manner described above for preparation of **16** from **15**. $[\alpha]_D^{25}=-31°$ (*c* 0.03, $CHCl_3$); the ^1H NMR spectrum was identical to that prepared from **15**.

3.6. Antifungal assay on TLC

EtOAc solutions containing 0.01–10 μg of test compound were spotted (5 mm in diameter) onto silica gel TLC plates (Merck, Silica gel 60 F_{254}). Conidia of *C. musae* strain no. 5501 were suspended in a Czapek-Dox medium at a density of 6.0×10^6 conidia/mL, and sprayed onto the TLC plates to completely wet the surface. The thin layers were incubated in a moist chamber at 23°C for 2 days in the dark. Zones of antifungal activity were detected as flat spots lacking mycelia.

3.7. Antifungal assay in aqueous solution

Conidia of *C. musae* strain no. 1679 were suspended in sterile H_2O at a density of 1.0×10^4 conidia/mL. A DMSO solution (25 μL) of test compound and Tween 20 (2.5 μL) were added to 5 mL of the conidia-suspension at final concentrations of 0.3–30 ppm. Tissue culture flasks (Iwaki, 75 cm^2, canted neck) containing the suspensions were placed at 23°C in the dark. After 24 h, 100 conidia were observed under a microscope (Olympus, IM). The inhibition ratio was defined as $[(A-B)/A]\times100$, where *A*=the number of conidia that germinated when H_2O containing 0.05% Tween 20 and 0.5% DMSO was used, and *B*=the number of conidia that germinated when test compound was used. The number *A* was 62–77.

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