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Phenylphenalenone-type Phytoalexins from Unripe Buñgulan Banana Fruit

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Fourteen phenylphenalenone-type phytoalexins (1–14), including three new compounds, were isolated from the peel of unripe *Musa acuminata* [AAA] cv. Buñgulan fruit which had been injured and then inoculated with conidia of *Colletotrichum musae*. These new phytoalexins were identified as (+)-*cis*-2,3-dihydro-2,3-dihydroxy-4-(4′-hydroxyphenyl)phenalen-1-one (12), 9-(3′,4′-dimethoxyphenyl)-2-methoxyphenalen-1-one (13) and 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one (14). The ratios of the relative intensities of the $[M]^+/[M-H]^+$ ions or $[M-H_2O]^+/[M-H_2O-H]^+$ ions in the EI mass spectra were applied to discriminate between 4- and 9-phenylphenalenones. An antifungal test on the phytoalexins showed that a phenolic hydroxyl group was essential for the activity.

Key words: banana; *Musa acuminata*; 1,8-naphthalenedicarboxylic acid; phenylphenalenone; phytoalexin

Anthracnose is a typical disease of banana (*Musa acuminata*) fruit, and is caused by infection by *Colletotrichum musae*.[1] The unripe fruit shows resistance to growth of the fungal hyphae, and the pathogen is quiescent until the fruit ripens, suggesting that the unripe fruit produces phytoalexin.[2,3] We found antifungal 2-(4′-hydroxyphenyl)-1,8-naphthalic anhydride (**1**) as a new phytoalexin, together with methyl 2-benzimidazole carbamate, an active form of benomyl, in the unripe fruit of cv. Grand Nain which had been injured and inoculated with conidia of *C. musae*.[4] However, there still remain unidentified phytoalexins in banana fruit.[4] We used unripe cv. Buñgulan fruit which had not been treated with fungicides to identify such phytoalexins. We describe here the structures of new phytoalexins isolated from the unripe fruit, and their antifungal activities. Determination of the position of the phenyl group in 4- and 9-phenylphenalenones by mass spectra will also be discussed.

The unripe cv. Buñgulan fruit was injured and then inoculated with a suspension of conidia of *C. musae* strain no. 1679. Autobiography of extracts from the peel with *C. musae* strain no. 5501, which is more sensitive than strain no. 1679, showed a broad antifungal zone between R_fs of 0.24 and 0.12 which was not detectable in extracts of the untreated fruit. Fourteen phenylphenalenones and their derivatives (**1–14**), shown in Fig. 1, with or without antifungal activity were isolated from the extracts. Nine compounds were identified as **1**,[4] anigorufone (**2**),[5] hydroxyanigorufone (**3**),[6,7] irenolone (**4**),[7] 2-hydroxy-4-(4′-methoxyphenyl)phenalen-1-one (**5**),[8] 2-methoxy-9-phenylphenalen-1-one (**6**),[9] (−)-*trans*-2,3-dihydro-2,3-dihydroxy-9-phenylphenalen-1-one (**7**),[9] (−)-*cis*-2,3-dihydro-2,3-dihydroxy-9-phenylphenalen-1-one (**8**),[9] and (−)-1,2-*trans*-2,3-*cis*-2,3-dihydro-1,2,3-trihydroxy-4-(4′-methoxyphenyl)phenalene (**9**)[6] by comparing their spectral data with those of an authentic sample and those described in the literature.

The spectral data of compounds **10** and **11**, with the exception of their UV spectra, were consistent with those of (+)-*cis*-2,3-dihydro-2,3-dihydroxy-4-(4′-methoxyphenyl)phenalen-1-one and (−)-*trans*-2,3-dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl) phenalen-1-one, respectively.[8,10] The UV spectrum reported by Luis *et al.* had an absorption maximum at a wavelength longer than 340 nm, suggesting that the samples were contaminated by **5** and **3** derived from **10** and **11**, respectively, by dehydration. Compound **10**, with a *cis* configuration, was so unstable that it partly changed to **5** during isolation by preparative TLC. Compounds **10** and **11** were changed to **5** and **3**, respectively, by acid treatment, although **11**, which had a *trans* configuration, was more resistant to dehydration than **10**. The other three compounds, **12**, **13** and **14**, were new phenylphenalenones.

The ^{1}H-NMR spectrum of compound **12** was similar to that of **10**, except for a singlet due to the 4′-*O*-methyl group, suggesting that **12** was a demethyl derivative of **10**. Its FAB mass spectrum showed an $[MH]^+$ ion at m/z 307. These results indicate that **12** was (+)-2,3-dihydro-2,3-dihydroxy-4-(4′-hydroxyphenyl) phenalen-1-one, and the structure was confirmed by converting **12** to **4** by an acid treatment. The coupling constant of 3.1 Hz between H-2 and H-3 was similar to that of **10**, so the relative configuration of **12** would be *cis*. The *cis* configuration is consistent with the ready dehydration of **12** to **4**.

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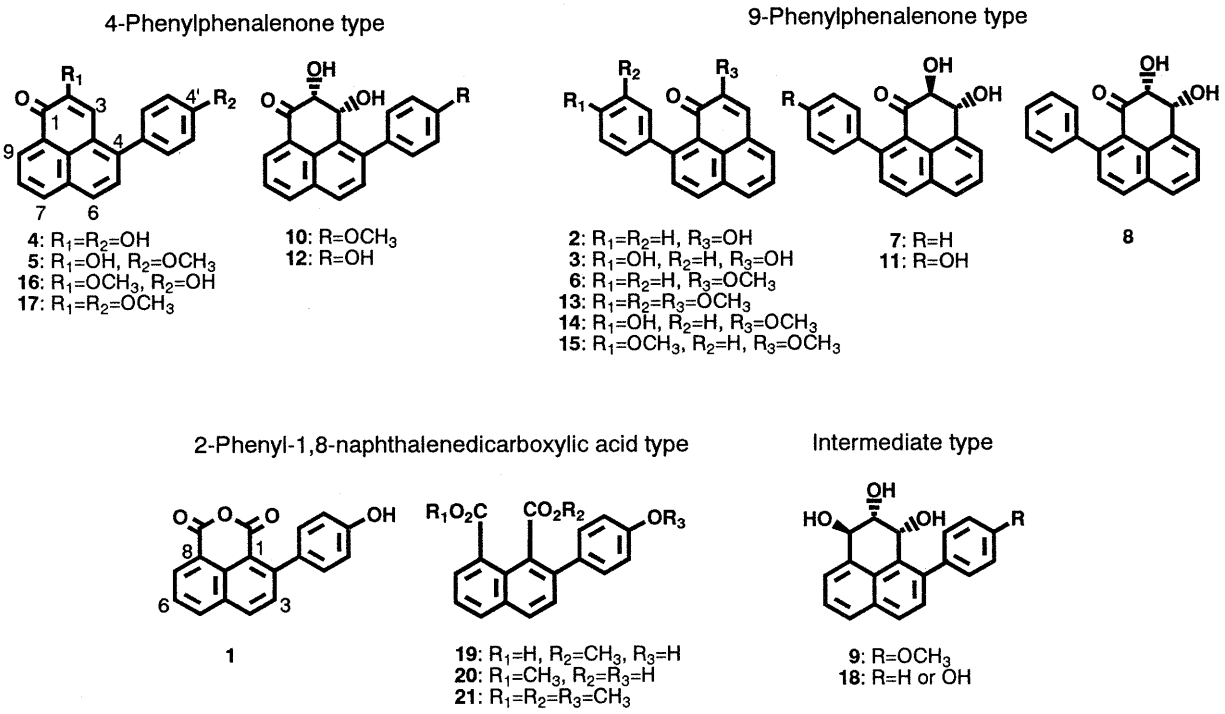

Fig. 1. Phytoalexins (**1–14**) Isolated from the Fruit of Buñgulan Banana, their Derivatives (**15–17** and **19–21**), and a Putative Biosynthetic Intermediate (**18**).

The relative configurations are shown.

Compound **13** showed signals of a 1,2,4-substituted benzene, a 2,4- or 2,9-substituted phenalen-1-one and three methoxy groups in the ^{1}H-NMR spectrum. One methoxy group was assigned to bind to C-2, since H-2 of phenalen-1-one was not apparent, suggesting that **13** was 2-methoxyphenalen-1-one substituted with a 2,4- or 3,4-dimethoxyphenyl group at C-4 or C-9. Signals of three protons on the dimethoxyphenyl group were observed at δ 6.82 (1H, dd, J=8.1 and 1.8 Hz), 6.88 (1H, d, J=8.1 Hz), and 6.99 (1H, d, J=1.8 Hz) ppm. The chemical shifts, δ 6.99 ppm of a doublet signal showing a meta coupling and δ 6.88 ppm of a doublet signal coupled only with a vicinal proton, mean that the phenyl group did not have a 2,4-dimethoxy but a 3,4-dimethoxy group since, in a 2,4-dimethoxyphenyl group, a doublet signal of H-3 showing meta coupling would have appeared at about δ 6.4 ppm owing to 2,4-methoxy groups that have a strong electron-donating effect,[11] and a doublet signal of H-6 coupling only with a vicinal proton would have appeared in a lower magnetic field than that of H-3. The position of the 3,4-dimethoxyphenyl group was deduced from the EI mass spectrum, since the amount was too small to measure the HMQC and HMBC spectra. In the EI mass spectra, the intensities of the $[M-H]^+$ or $[M-H_2O-H]^+$ ions of 9-phenylphenalenones **2**, **3**, **6–8** and **11** were higher than those of the $[M]^+$ or $[M-H_2O]^+$ ions, while the intensities of the $[M-H]^+$ or $[M-H_2O-H]^+$ ions of 4-phenylphenalenones **4**, **5**, **10** and **12** were lower than those of the $[M]^+$ or $[M-H_2O]^+$ ions, as summarized in Table. The high intensity of the $[M-H]^+$ or $[M-H_2O-H]^+$ ions of the 9-phenylphenalenones can be explained by the ready formation of stable $[M-H]^+$ or $[M-H_2O-H]^+$ ions as shown in Fig. 2.[12] A bond was formed between the 1-oxygen and C-2′ of the $[M]^+$ or $[M-H_2O]^+$ ions, and a hydrogen radical at C-2′ of **22** was eliminated to give a stable $[M-H]^+$ or $[M-H_2O-H]^+$ ion (**23**) with a resonance structure. The ratio between the relative intensities of the $[M]^+$ and $[M-H]^+$ ions or of the $[M-H_2O]^+$ and $[M-H_2O-H]^+$ ions could be applied to discriminate between 4- and 9-phenylphenalenones. A similar rule has been used to discriminate between 7- and 9-phenylphenalenones from *Haemodorum corymbosum*.[13] In the mass spectrum of **13**, the relative intensity of the $[M-H]^+$ ion was higher than that of the $[M]^+$ ion at m/z 332, indicating that **13** was 9-(3′,4′-dimethoxyphenyl)-2-methoxyphenalen-1-one.

The ^{1}H-NMR spectrum of compound **14** showed a singlet of three protons at δ 3.84 ppm, in addition to the signals of **3** excepting a hydroxyl proton, suggesting that **14** was a monomethyl ether of **3**. Two hydroxyl protons of **3** at δ 7.77 and 8.46 ppm were assigned to 2- and 4′-hydroxyl groups, respectively, by HMQC and HMBC spectra. In the spectrum of **14**, the former hydroxyl proton was not apparent, while the latter hydroxyl proton was observed at δ 8.37 ppm; compound **14**, therefore, was identified as 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one. The EI mass spectrum of **14** also showed higher intensity of the $[M-H]^+$ ion than of the $[M]^+$ ion. Treatment of **3** with diazomethane gave **14** first, which had a smaller R_f value than that of 3, and then gave 2-methoxy-9-(4′-methoxyphenyl)phenalen-1-one (**15**). The reactivities of the two hydroxyl groups of **4** were the same as those of **3**, and treatment of **4** with dia-

Table Relative Intensities of $[M]^+$ or $[M-H_2O]^+$ Ions, and $[M-H]^+$ or $[M-H_2O-H]^+$ Ions of 4- and 9-Phenylphenalenones in EI Mass Spectra

Type	Compound	Mass weight	Relative intensity (%)	
			$[M]^+$ or $[M-H_2O]^+$ ion	$[M-H]^+$ or $[M-H_2O-H]^+$ ion
4-Phenylphenalenones				
	4	288	100	29
	5	302	100	14
	10	320	82	0
	12*	306	100	26
9-Phenylphenalenones				
	2	272	50	100
	3	288	53	100
	6	286	53	100
	7*	290	65	100
	8*	290	61	100
	11*	306	57	100
	13	332	34	47
	14	302	60	100

* For these compounds, the relative intensities of the $[M-H_2O]^+$ and $[M-H_2O-H]^+$ ions are shown, since they showed hardly any $[M]^+$ or $[M-H]^+$ ions.

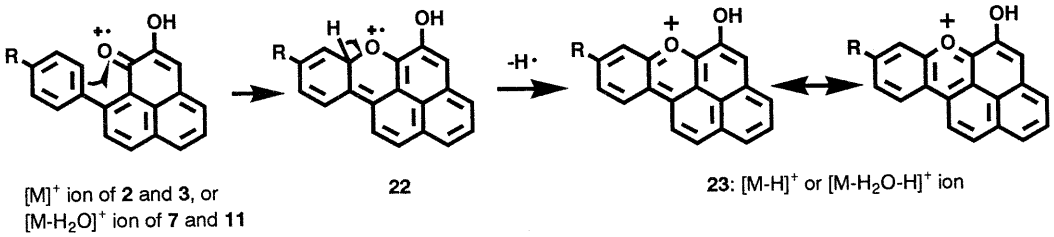

Fig. 2. Formation of $[M-H]^+$ or $[M-H_2O-H]^+$ Ions from 9-Phenylphenalenones in EIMS.
R=H or OH or OCH_3

zomethane gave first **16**, and then **17**. The 2-*O*-methyl group of **16** was unstable and easily eliminated, although the reason for this is unknown. This is in agreement with the lack of isolation of the 2-methoxy derivatives of 4-phenylphenalenones.

These phenylphenalenones can be classified into four types: 4-phenylphenalenone, 9-phenylphenalenone, 2-phenyl-1,8-naphthalenedicarboxylic acid, and an intermediate type, as shown in Fig. 1. The relative configurations between H-2 and H-3 of the 2,3-dihydro-2,3-dihydroxyl derivatives of 4-phenylphenalenones **10** and **12**, and of 9-phenylphenalenones **7** and **11** were *cis* and *trans*, respectively. Luis *et al.* have proposed that phenylphenalenones in banana plants are biosynthesized via 1,2-*trans*-2,3-*cis*-2,3-dihydro-1,2,3-trihydroxyphenylphenalene like **9** and putative intermediate **18**, which is converted to 4-phenylphenalenones and 9-phenylphenalenones after oxidation at C-1 and C-3, respectively, and subsequent dehydration;[6] hydroxyl and methoxy groups of the phenyl group seem to have been introduced before formation of the 2,3-dihydro-1,2,3-trihydroxyphenalene moiety. However, the occurrence of **8**, *cis*-2,3-dihydro-2,3-dihydroxy-9-phenylphenalenone, is not consistent with this pathway. Compound **8** may have been an artifact derived from **7** by epimerization through an enol form of the 1-carbonyl group, or the phenylphenalenones of banana fruit might be biosynthesized via another pathway. Compound **1** was found only in fruit, while other phenylphenalenones **2–11** were also isolated from rhizome,[6,8-10] suggesting that the enzyme synthesizing **1**, probably by eliminating C-2 of the phenylphenalenones, is produced only in fruit.

Compound **1** exposed to methanol gave monomethyl esters **19** and **20** of 2-(4′-hydroxyphenyl)-1,8-naphthalenedicarboxylic acid. The position of the esterified carboxyl group was deduced by comparing the chemical shift of the methoxy protons in the ^{1}H-NMR spectrum with that of a trimethyl derivative (**21**) of **1**. The signals of two carbomethoxy groups of **21** appeared at δ 3.51 and 3.85 or 3.86 ppm, the former signal being assigned to the 1-carbomethoxy group, since the up-field shift could be explained by the shielding effect of the 4′-methoxyphenyl group at C-2. The dihedral angles between the phenyl ring and the phenalene moiety of **2** and **3** were 51°[9] and 60°,[6] respectively, suggesting that the 4′-methoxyphenyl and the naphthalene rings of **21** had 50-60° of a dihedral angle to show the shielding effect. The chemical shifts of δ 3.50 and 3.79 ppm of the carbomethoxy groups of **19** and **20**, respectively, indicate that these compounds were 2-(4′-hydroxyphenyl)-1-methoxycarbonyl-8-naphthalenecarboxylic acid* and 2-(4′-hydroxyphenyl)-8-methoxycarbonyl-1-naphthalene-

* The numbering system of the naphthalene ring was not changed for clarity of the position of the esterified carboxyl and 4′-hydroxyphenyl groups, although the IUPAC name of **19** is 7-(4′-hydroxyphenyl)-8-methoxycarbonyl-1-naphthalenecarboxylic acid.

carboxylic acid, respectively. The ratio of 1:3 of **19** and **20** suggests that methanolysis of **1** was affected by steric hindrance due to the 4′-hydroxyphenyl group.

The antifungal activities of compounds **1–17** and **19–21** were examined by TLC autobiography. Compounds **1**, **19** and **20**, compound **4**, and compounds **3** and **12** showed antifungal activity at minimum amounts of 0.3 μg, 1.0 μg, and 3.0 μg, respectively, while the other compounds were inactive at 10 μg. This finding indicates that a phenolic hydroxyl group was essential for the activity of these three types of compounds. The lability of the anhydride group of **1** seems to have been important for the high level of activity of 2-phenyl-1,8-naphthalenedicarboxylic acid-type compounds, and compounds **19** and **20** might be active after their conversion to **1**. The higher activity of **4** than that of **3** indicates that the 4-phenylphenalenone-type compounds would be more active than the 9-phenyphenalenone-type compounds. The activity of **12**, which has a *cis* configuration, would have been due to the formation of **4** by ready dehydration since **11**, which has a *trans* configuration, was inactive.

Experimental

^{1}H- and ^{13}C-NMR, HMQC and HMBC spectra were recorded with TMS as an internal standard by using Bruker ARX500 and AC300 apparatus. Mass spectra were obtained with a Jeol JMS-DX300/DA5000 mass spectrometer. UV and IR spectra were recorded with Shimadzu UV-2200AI and FTIR-8100AI spectrometers, respectively. Optical rotation was measured by a Jasco DIP-1000 polarimeter.

Materials. M. acuminata [AAA] cv. Buñgulan was cultivated on Negulos Island in the Philippines, and unripe fruit was imported by Alter Trade Japan Inc., Tokyo, Japan. *C. musae* (Berk. & 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 cultured on a potato-sucrose-agar medium at 23°C in the dark.

Injury and Inoculation. Unripe banana fruit was washed with water, wiped with 70% ethanol and rubbed with sandpaper (G-60 or 80). Conidia of *C. musae* strain no. 1679 were suspended in sterilized water at a density of 6×10^6 ml^{-1}. The injured fruit was soaked in the suspension in a plastic bag for 10 sec, and incubated in the plastic bag at 24°C for 5–7 days in the dark. The peel of treated fruit was used for isolating the compounds. Control fruit was incubated under the same conditions without treatment.

Autobiography. Aliquots of an ethyl acetate solution of each sample were applied to thin-layer silica gel plates, and developed with chloroform-methanol (39:1) until 8 cm. After drying the solvent, conidia of *C. musae* strain no. 5501 suspended in a Czapek-Dox medium at a density of 10^6 ml^{-1} were sprayed on to the thin-layer plates, which were then incubated in a moist chamber at 23°C for 3 days in the dark. Antifungal compounds were detected as zones lacking aerial mycelia and by the absence of hyphae turning brown on exposure to iodine vapor.

Isolation of compounds 1, 3, 4, 10 and 12. Peel (984 g) from 28 fruits was soaked in 1.3 l of ethyl acetate for 3 days. The solution was filtered, washed with water three times, and dried over sodium sulfate. The ethyl acetate extract (2.07 g) was applied to a silica gel (100 g) column eluted with toluene-ethyl acetate.

The fraction eluted with 20% ethyl acetate gave a yellow solid, which crystallized from acetonitrile to give 7.0 mg of **1** as yellow needles, mp 275°C. The concentrated mother liquor (92 mg) was applied to a silica gel (10 g) column eluted with toluene-ethyl acetate. The fractions eluted with 14% and 16% ethyl acetate were combined, concentrated, and chromatographed in an ODS (AM120-S50, 17 g; YMC) column with methanol-water. The material eluted with 70% methanol, and the second material eluted from another ODS column with 70% methanol which is described later, were combined and applied to an ODS (AM120-S50, 17 g) column eluted with 60% methanol. The fraction of 190–250 ml was separated by silica gel TLC with *n*-hexane-chloroform-methanol (15:5:1) and developed five times, the bands at R_f 0.5 and R_f 0.3 giving 4.0 mg of **3** as a red solid and 2.1 mg of **4** as dark red needles, respectively.

The fraction eluted with 50% ethyl acetate from the first silica gel column was chromatographed in an ODS (AM120-S50, 17 g) column with methanol-water. The material (4.0 mg) eluted with 60% methanol was applied to a silica gel (0.8 g) column eluted with toluene-ethyl acetate. The fractions eluted with 30% and 50% ethyl acetate were combined, concentrated, and purified by HPLC in an AQ-311 column (ODS, 6 × 100 mm; YMC), eluting with methanol-water (1:1) at a flow rate of 1.0 ml min^{-1} with detection at 280 nm. The material eluted at t_R 17.5 min was collected and concentrated to give 1.8 mg of **12** as a yellowish-white powder. The first material (10 mg) eluted from the ODS column with 70% methanol was concentrated and purified by HPLC with a μBondasphere 5 μ C18-100 Å column (ODS, 19 × 150 mm; Waters), eluting with methanol-water (6:4) at a flow rate of 5.0 ml min^{-1} with detection at 254 nm. The material with t_R 21.4 min was collected and concentrated to give 1.2 mg of **10**. The second material (9 mg) eluted from the ODS column with 70% methanol was combined with the material eluted with 70% methanol that had already been described.

Isolation of compounds 2, 5, 7 and 11. Peel (264 g) was extracted with 450 ml of ethyl acetate, and the extracted material (616 mg) was applied to a silica gel (60 g) column eluted with toluene-ethyl acetate. The material eluted with 10% ethyl acetate was subjected to silica gel TLC with *n*-hexane-ethyl acetate-methanol (15:5:1). The material at R_f 0.37 was extracted with ethyl acetate to give 1.9 mg of **5**. The material at R_f 0.48 was purified twice by preparative silica gel TLC, using *n*-hexane-chloroform-methanol (15:5:1). The material at R_f 0.61 was extracted with ethyl acetate, and the ethyl acetate solu-

tion was concentrated to give 0.5 mg of **2**. The material eluted with 50% ethyl acetate was chromatographed in an SH-342-5 column (ODS, 19×150 mm; YMC) with methanol-water (7:3) at a flow rate of 7.5 ml min^{-1} with detection at 254 nm. The material with t_R 14.0 min was collected and concentrated to give 1.2 mg of **7** as a yellowish-white powder. The material with t_R 9.0 min was purified twice by preparative HPLC with an SH-342-5 column eluted with acetonitrile-water (65:35) at a flow rate of 7.5 ml min^{-1} with detection at 254 nm. Collection of the material at t_R 16.4 min gave 0.5 mg of **11** as yellow needles, mp 228–231°C.

Isolation of compounds 6, 8, 9, 13 and 14. The material (478 mg) extracted from peel (86 g) with 150 ml of ethyl acetate was applied to a silica gel (50 g) column eluted with toluene-ethyl acetate. The fraction eluted with 50% ethyl acetate was subjected to silica gel TLC, using *n*-hexane-chloroform-methanol (5:15:1) as the eluent. The UV-absorbent band at R_f 0.82 was extracted to give 0.3 mg of **6**. The UV-absorbent material at R_f 0.50 was purified twice by preparative silica gel TLC developed with *n*-hexane-ethyl acetate-methanol (10:10:1) to give 0.2 mg of **8**. The fraction eluted with 100% ethyl acetate was purified by preparative silica gel TLC with *n*-hexane-chloroform-methanol (5:15:1). The UV-absorbent bands at R_fs 0.36 and 0.24 were extracted with ethyl acetate to give 0.1 mg of **13** as a yellow solid and 1.2 mg of **14** as an orange powder, respectively. The UV-absorbent material at R_f 0.11 was purified twice by silica gel TLC, using *n*-hexane-ethyl acetate-methanol (5:15:1, R_f of 0.44) as the eluent, to give 0.2 mg of **9**.

2-(4′-Hydroxyphenyl)-1,8-naphthalic anhydride (1). IR ν_{max} (KBr) cm^{-1}: 3500, 1760 (s), 1725 (s), 1610, 1585, 1565, 1495, 1360, 1275, 1200, 1005 (s), 745; NMR δ_C (125 MHz, acetone-d_6): 116.3 (C-3′ and C-5′), 116.6 (C-1), 120.7 (C-8), 128.1 (C-6), 131.6 (C-2′ and C-6′), 132.4 (C-4a), 132.6 (C-8a), 133.1 (C-1′), 133.2 (C-3), 134.1 (C-7), 135.5 (C-4), 136.7 (C-5), 151.0 (C-2), 159.1 (C-4′), 160.2 (C-9), 162.2 (C-10); the data in the literature[4] were partially in error. See ref. 4 for the other spectral data.

Anigorufone (2). EIMS (70 eV) *m/z* (rel. int.): 272 $[M]^+$ (50), 271 $[M-H]^+$ (100), 242 (5), 215 (7), 213 (8), 136 (9), 113 (6), 107 (6). See ref. 5 for the other spectral data.

Hydroxyanigorufone (3). NMR δ_C (125 MHz, acetone-d_6): 113.2 (C-3), 116.1 (C-3′ and C-5′), 125.0 (C-9a), 126.4 (C-9b), 128.1 (C-5), 130.3 (C-3a), 130.6 (C-6), 131.1 (C-2′ and C-6′), 131.3 (C-4), 132.7 (C-6a), 132.9 (C-8), 134.8 (C-1′), 136.5 (C-7), 150.1 (C-9), 151.8 (C-2), 158.4 (C-4′), 181.2 (C-1); the data in the literature[14] were partially in error. EIMS (70 eV) *m/z* (rel. int.): 288 $[M]^+$ (53), 287 $[M-H]^+$ (100), 271 (19), 259 (6), 231 (3), 213 (5), 202 (12). See ref. 7 for the other spectral data.

Irenolone (4). EIMS (70 eV) *m/z* (rel. int.): 288 $[M]^+$ (100), 287 $[M-H]^+$ (29), 271 (43), 270 (47), 260 (16), 242 (23), 231 (28), 213 (10), 202 (34), 176 (6), 121 (12), 107 (10), 101 (17). See ref. 7 for the other spectral data.

2-Hydroxy-4-(4′-methoxyphenyl)phenalen-1-one (5). EIMS (70 eV) *m/z* (rel. int.): 302 $[M]^+$ (100), 301 $[M-H]^+$ (14), 287 (18), 284 $[M-H_2O]^+$ (24), 271 (41), 259 (17), 202 (47), 151 (16), 106 (20), 101 (47). See ref. 8 for the other spectral data.

2-Methoxy-9-phenylphenalen-1-one (6). EIMS (70 eV) *m/z* (rel. int.): 286 $[M]^+$ (53), 285 $[M-H]^+$ (100), 270 (14), 255 (14), 242 (19), 226 (8), 213 (15), 121 (13), 107 (13). See ref. 9 for the other spectral data. Methylation of **2** (0.2 mg) in 0.2 ml of methanol with an ether solution of diazomethane for 1 h gave **6**.

(−)-trans-2,3-Dihydro-2,3-dihydroxy-9-phenylphenalen-1-one (7). $[\alpha]_D^{26}$ −142° (*c* 0.10, methanol); EIMS (70 eV) *m/z* (rel. int.): 272 $[M-H_2O]^+$ (65), 271 $[M-H_2O-H]^+$ (100), 242 (8), 215 (10), 213 (10), 189 (4), 136 (12), 121 (5), 113 (10), 107 (9), 95 (9); FABMS (3-nitrobenzylalcohol) *m/z* (rel. int.): 313 $[MNa]^+$ (45), 291 $[MH]^+$ (34), 273 $[MH-H_2O]^+$ (23). See ref. 9 for the other spectral data. Dehydration of **7** (0.3 mg) with *p*-toluenesulfonic acid in 1 ml of methylenechloride at 45°C for 15 min gave 0.2 mg of **2**.

(−)-cis-2,3-Dihydro-2,3-dihydroxy-9-phenylphenalen-1-one (8). $[\alpha]_D^{24}$ −46° (*c* 0.01, methanol); EIMS (70 eV) *m/z* (rel. int.): 290 $[M]^+$ (25), 272 $[M-H_2O]^+$ (61), 271 $[M-H_2O-H]^+$ (100), 244 (34), 243 (37), 215 (40), 202 (15), 149 (20). See ref. 9 for the other spectral data.

(−)-1,2-trans-2,3-cis-2,3-Dihydro-1,2,3-trihydroxy-4-(4′-methoxyphenyl)phenalene (9). $[\alpha]_D^{23}$ −25° (*c* 0.02, methanol). See ref. 6 for the other spectral data.

(+)-cis-2,3-Dihydro-2,3-dihydroxy-4-(4′-methoxyphenyl)phenalen-1-one (10). $[\alpha]_D^{25}$ +25° (*c* 0.28, methanol); UV λ_{max} (methanol) nm (ε): 218 (26,000), 228 (28,000), 245 (19,000), 273 (12,000), 322 (5,200); EIMS (70 eV) *m/z* (rel. int.): 320 $[M]^+$ (82), 302 $[M-H_2O]^+$ (81), 284 (15), 273 (100), 271 (22), 259 (15), 245 (19), 231 (12), 215 (14), 202 (43), 189 (34). See ref. 8 for the other spectral data. Compound **10** (0.2 mg) was dissolved in 0.2 ml of methylenechloride, and 3 mg of *p*-toluenesulfonic acid was added to the solution. After standing at 45°C for 15 min, the solution was concentrated, and the residue was dissolved in 1 ml of toluene before being chromatographed in a Sep-pak cartridge of silica gel (Waters). The material eluted with 10% ethyl acetate was dried to give 0.2 mg of **5**.

(−)-trans-2,3-Dihydro-2,3-dihydroxy-9-(4′-hydroxyphenyl)phenalen-1-one (11). $[\alpha]_D^{23}$ −158° (*c* 0.05, methanol); UV λ_{max} (methanol) nm (ε): 219 (35,800), 252 (23,700), 288 (11,000), 336 (6,900); EIMS (70 eV) *m/z* (rel. int.): 288 $[M-H_2O]^+$ (57), 287 $[M-H_2O-H]^+$ (100), 271 (22), 259 (29), 231 (12), 202 (18), 149 (13), 121 (12); FABMS (glycerol) *m/z* (rel. int.): 329 $[MNa]^+$ (62), 307 $[MH]^+$ (90), 289 $[MH-H_2O]^+$ (75), 271 [MH-

$2H_2O]^+$ (62). See ref. 10 for the other spectral data. Compound **11** (50 μg) was treated with *p*-toluenesulfonic acid by the same method as that used for **10** to give **3**.

(+)-cis-2,3-Dihydro-2,3-dihydroxy-4-(4'-hydroxyphenyl)phenalen-1-one (12). $[\alpha]_D^{25}+44°$ (*c* 0.18, methanol); UV λ_{max} (methanol) nm (ε): 219 (24,000), 229 (25,000), 245 (17,000), 273 (11,000), 325 (4,600); NMR δ_H (500 MHz, acetone-d_6): 4.51 (1H, s, OH-3 or 2), 4.57 (1H, s, OH-2 or 3), 4.80 (1H, d, J=3.1 Hz, H-3), 5.29 (1H, d, J=3.1 Hz, H-2), 6.99 (2H, d, J=8.6 Hz, H-3' and H-5'), 7.55 (2H, d, J=8.6 Hz, H-2' and H-6'), 7.62 (1H, d, J=8.5 Hz, H-5), 7.70 (1H, dd, J=8.1 and 7.0 Hz, H-8), 8.07 (1H, d, J=8.5 Hz, H-6), 8.14 (1H, dd, J=7.0 and 0.9 Hz, H-7), 8.27 (1H, dd, J=8.1 and 0.9 Hz, H-9), 8.60 (1H, s, OH-4'); EIMS (70 eV) *m/z* (rel. int.): 288 $[M-H_2O]^+$ (100), 287 $[M-H_2O-H]^+$ (26), 271 (41), 270 (40), 260 (14), 242 (18), 231 (24), 202 (26), 121 (13), 101 (17); FABMS (glycerol) *m/z* (rel. int.): 307 $[MH]^+$ (12), 287 $[MH-H_2O]^+$ (75). Compound **12** (0.2 mg) was dehydrated with *p*-toluenesulfonic acid in the same manner as that used for **10** to give **4**.

9-(3',4'-Dimethoxyphenyl)-2-methoxyphenalen-1-one (13). UV λ_{max} (methanol) nm (ε): 258 (17,000), 270 (16,000), 365 (5,000), 410 (4,600); NMR δ_H (300 MHz, acetone-d_6): 3.58 (3H, s, OMe-3'), 3.836, (3H, s, OMe-4' or 2), 3.842 (3H, s, OMe-2 or 4'), 6.82 (1H, dd, J=8.1 and 1.8 Hz, H-6'), 6.88 (1H, d, J=8.1 Hz, H-5'), 6.99 (1H, d, J=1.8 Hz, H-2'), 7.11 (1H, s, H-3), 7.61 (1H, d, J=8.4 Hz, H-8), 7.63 (1H, dd, J=8.9 and 7.1 Hz, H-5), 7.82 (1H, dd, J=7.1 and 1.2 Hz, H-4), 8.01 (1H, dd, J=8.9 and 1.2 Hz, H-6), 8.28 (1H, d, J=8.4 Hz, H-7); EIMS (70 eV) *m/z* (rel. int.): 332 $[M]^+$ (34), 331 $[M-H]^+$ (47), 317 (19), 316 (14), 315 (12), 301 (12), 289 (5), 256 (6), 221 (12), 177 (9), 147 (15), 133 (32), 57 (100).

9-(4'-Hydroxyphenyl)-2-methoxyphenalen-1-one (14). UV λ_{max} (methanol) nm (ε): 237 (9,700), 260 (11,100), 267 (10,900), 308 (2,600), 348 (3,400), 368 (4,600), 404 (4,000), 411 (4,100); NMR δ_H (300 MHz, acetone-d_6): 3.84 (3H, s, OMe-2), 6.88 (2H, d, J=8.7 Hz, H-3' and H-5'), 7.11 (1H, s, H-3), 7.23 (2H, d, J=8.7 Hz, H-2' and H-6'), 7.58 (1H, d, J=8.3 Hz, H-8), 7.62 (1H, dd, J=8.1 and 7.2 Hz, H-5), 7.81 (1H, dd, J=7.2 and 1.1 Hz, H-4), 8.00 (1H, dd, J=8.1 and 1.1 Hz, H-6), 8.28 (1H, d, J=8.3 Hz, H-7), 8.37 (1H, s, OH-4'); EIMS (70 eV) *m/z* (rel. int.): 302 $[M]^+$ (60), 301 $[M-H]^+$ (100), 286 (13), 285 (19), 271 (13), 258 (15), 202 (13), 151 (7), 129 (6), 101 (12).

2-Methoxy-9-(4'-methoxyphenyl)phenalen-1-one (15). Compound **3** (1.2 mg) dissolved in 2 ml of acetone-methanol (1:1) was methylated with an ethereal solution of diazomethane for 10 min. The product was subjected to silica gel TLC with *n*-hexane-chloroform-methanol (10:10:1) as the eluent. The UV-absorbent bands with R_fs 0.1 and 0.6 were extracted with ethyl acetate to give 0.6 mg of **14** and 0.6 mg of **15**, respectively. Compound **15**. UV λ_{max} (methanol) nm (ε): 239 (11,900), 261 (12,800), 266 (12,200), 312 (3,800), 347 (4,200), 367 (5,000), 408 (3,800); NMR δ_H (300 MHz, acetone-d_6): 3.84 (3H, s, OMe-2 or 4'), 3.86 (3H, s, OMe-4' or 2), 6.97 (2H, d, J=8.8 Hz, H-3' and H-5'), 7.12 (1H, s, H-3), 7.30 (2H, d, J=8.8 Hz, H-2' and H-6'), 7.58 (1H, d, J=8.3 Hz, H-8), 7.63 (1H, dd, J=8.1 and 7.1 Hz, H-5), 7.82 (1H, dd, J=8.1 and 1.0 Hz, H-4), 8.01 (1H, dd, J=7.1 and 1.0 Hz, H-6), 8.29 (1H, d, J=8.3 Hz, H-7); EIMS (70 eV) *m/z* (rel. int.): 316 $[M]^+$ (62), 315 $[M-H]^+$ (100), 301 (29), 300 (9), 286 (12), 285 (42), 272 (12), 242 (10), 229 (10), 213 (10), 202 (14), 200 (11), 158 (6).

4-(4'-Hydroxyphenyl)-2-methoxyphenalen-1-one (16) and 2-methoxy-4-(4'-methoxyphenyl)phenalen-1-one (17). Compound **4** (0.5 mg) dissolved in 1 ml of methanol was methylated with an ethereal solution of diazomethane for 1 min to give 0.3 mg of **16**. NMR δ_H (300 MHz, acetone-d_6): 3.77 (3H, s, OMe-2), 7.07 (2H, d, J=8.6 Hz, H-3' and H-5'), 7.20 (1H, s, H-3), 7.46 (2H, d, J=8.6 Hz, H-2' and H-6'), 7.63 (1H, d, J=8.5 Hz, H-5), 7.87 (1H, dd, J=7.9 and 7.4 Hz, H-8), 8.08 (1H, d, J=8.5 Hz, H-6), 8.39 (1H, dd, J=7.9 and 1.1 Hz, H-7), 8.64 (1H, dd, J=7.4 and 1.1 Hz, H-9); EIMS (70 eV) *m/z* (rel. int.): 302 $[M]^+$ (100), 301 $[M-H]^+$ (39), 285 (8), 273 (93), 272 (16), 256 (12), 255 (13), 244 (20), 231 (19), 202 (33), 135 (17), 121 (22), 101 (37). Further methylation of **16** with an ethereal solution of diazomethane gave **17**. UV λ_{max} (methanol) nm (ε): 229 (33,000), 243 (26,000), 263 (20,000), 316 (7,600), 339 (6,500), 400 (5,900); NMR δ_H (500 MHz, acetone-d_6): 3.76 (3H, s, OMe-2), 3.92 (3H, s, OMe-4'), 7.16 (2H, d, J=8.9 Hz, H-3' and H-5'), 7.17 (1H, s, H-3), 7.55 (2H, d, J=8.9 Hz, H-2' and H-6'), 7.63 (1H, d, J=8.5 Hz, H-5), 7.87 (1H, dd, J=8.1 and 7.3 Hz, H-8), 8.09 (1H, d, J=8.5 Hz, H-6), 8.39 (1H, dd, J=8.1 and 1.1 Hz, H-7), 8.65 (1H, dd, J=7.3 and 1.1 Hz, H-9); EIMS (70 eV) *m/z* (rel. int.): 316 $[M]^+$ (100), 315 $[M-H]^+$ (35), 301 (8), 287 (76), 286 (22), 271 (12), 243 (11), 215 (15), 202 (40), 101 (16).

2-(4'-Hydroxyphenyl)-1-methoxycarbonyl-8-naphthalenecarboxylic acid (19) and 2-(4'-hydroxyphenyl)-8-methoxycarbonyl-1-naphthalenecarboxylic acid (20). Compound **1** (3 mg) dissolved in 2 ml of methanol was kept at −20°C for 40 days. After being concentrated, the material was chromatographed in an AQ-311 column with methanol-acetic acid-water (60:0.1:40) at a flow rate of 1.0 ml min^{-1} with detection at 254 nm, and the material with t_R 6.5 min was purified twice by preparative HPLC with a μBondasphere 5 μ C18-100 Å column eluted with methanol-acetic acid-water (60:0.0025:40) at a flow rate of 5.0 ml min^{-1} with detection at 254 nm. The materials with t_R 23.5 min and 25.0 min were separately collected and concentrated to give 0.2 mg of **19** and 0.7 mg of **20**, respectively, as a white powder. Compound **19**. UV λ_{max} (methanol) nm (ε): 230 (27,000), 268 (10,000), 312 (4,500), 330 (2,800); NMR δ_H (500 MHz, acetone-d_6): 3.50 (3H, s, CO_2Me-1), 6.87 (2H, d, J=8.4 Hz, H-3' and H-5'), 7.13 (2H, d, J=8.4 Hz, H-2' and H-6'), 7.46 (1H, d, J=8.4 Hz, H-3), 7.54 (1H, dd, J=7.0 and 6.9 Hz, H-6), 7.90 (1H, d, J=6.9

Hz, H-5), 8.03 (1H, d, J=7.0 Hz, H-7), 8.04 (1H, d, J=8.4 Hz, H-4). Compound **20**. UV λ_{max} (methanol) nm (ε): 230 (27,000), 269 (11,000), 314 (5,400), 332 (3,500); NMR δ_H, (500 MHz, acetone-d_6): 3.79 (3H, s, CO_2Me-8), 6.89 (2H, d, J=8.5 Hz, H-3′ and H-5′), 7.26 (2H, d, J=8.5 Hz, H-2′ and H-6′), 7.52 (1H, d, J=8.3 Hz, H-3), 7.60 (1H, dd, J=8.4 and 7.2 Hz, H-6), 7.88 (1H, d, J=7.2 Hz, H-5), 8.08 (1H, d, J=8.4 Hz, H-4), 8.13 (1H, d, J=8.3 Hz, H-7).

Dimethyl 2-(4′-methoxyphenyl)-1,8-naphthalenedicarboxylate (21). An ethereal solution of diazomethane was added to a methanol solution of **1** (0.9 mg) and kept at room temperature for 1.5 h. The solutions were concentrated to give **21** as a colorless solid. NMR δ_H (500 MHz, acetone-d_6): 3.51 (3H, s, CO_2Me-1), 3.85 (3H, s, CO_2 Me-8 or OMe-4′), 3.86 (3H, s, OMe-4′ or CO_2Me-8), 7.03 (2H, d, J=8.7 Hz, H-3′ and H-5′), 7.28 (2H, d, J=8.7 Hz, H-2′ and H-6′), 7.57 (1H, d, J=8.4 Hz, H-3), 7.63 (1H, dd, J=8.1 and 7.1 Hz, H-6), 7.91 (1H, dd, J=7.1 and 1.1 Hz, H-5), 8.14 (1H, d, J=8.4 Hz, H-4), 8.16 (1H, dd, J=8.1 and 1.1 Hz, H-7); EIMS (70 eV) m/z (rel. int.): 350 $[M]^+$ (100), 319 (75), 291 (45), 276 (22), 261 (10), 233 (8), 189 (12).

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