Pergamon

Tetrahedron, Vol. 53, No. 24, pp. 8249-8256, 1997
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0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00490-0

Phenylphenalenonic Phytoanticipins. New Acenaphtylene and Dimeric Phenylphenalenones from the Resistant *Musa* Selected Hybrid SH-3481

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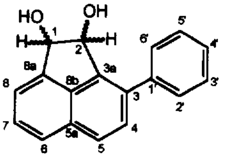
Abstract:: A new acenaphtylene derivative and two new dimeric phenylphenalenones has been isolated from healthy rhizomes of *Musa* selected hybrid resistant cultivar SH-3481. The structures of the new substances were elucidated using spectroscopy data. The chemical shift for all the hydrogen and carbon atoms in the substances were unambiguously established by mono and bi-dimensional, homo and heteronuclear NMR experiments. Seven previously reported phenylphenalenone type phytoalexins were also now isolated as constitutive natural antibiotics and so as phytoanticipins from this resistant banana cultivar, suggesting that this type of phytoalexins play an important role as part of the resistance mechanisms to fungal diseases in this important alimentary resource botany family. © 1997 Elsevier Science Ltd.

Compounds with a phenalenone nucleus are rare in Nature, but some of such substances have been reported in plants and microorganisms[1,2]. The plant phenalenones posess a side phenyl ring on C-9 and have been found in some species of the *Haemodoraceae* family.

The phytoalexins, on the other hand, are a group of natural products defined by their physiological rather than their structural features. These compounds are produced *de novo* by some plant organs when provoked by biotic, physical or chemical agents[3]. The phytoalexins represent a structurally diverse group of compounds. Depending on the plant species examined, the groups of compounds identified as phytoalexins includes diterpenes, sesquiterpenes, furanocumarins, isoflavonoids, polyacetylenes and many more. However, they have in common both antimicrobial activity and accumulation in response to infection. Emphasis has been rightly placed on the molecular biology and genetic of interactions between plants and bacteria or virus.

This is consistent with present interest in and knowledge gained from studies with these organisms. In contrast, molecular work on pathogenic fungi is still in its infancy[4] . Previously we had described the isolation, characterization and synthesis[5-9] of the first examples of a new type of phytoalexins structurally based on a phenalenone skeleton, from leaves and fruit peels of *Musa paradisiaca* [M. acuminata (AAA), Grand Nain], elicited by kanamycin or by *Mycosphaerella fijiensis* (casual agent of Black Sigatoka) a pathogenic fungus which attacks banana leaves and greatly reduces their growth. Structurally similar compounds with a *p*-hydroxy-phenyl substituent attached to the C-4 or C-9 carbon atom of a perinaphthenone nucleus were obtained[6] from rhizomes infected with the pathogenic fungus *Fusarium oxysporum* f. sp.*cubense* race 4, which causes the Panama's disease in banana plants. This substances were not present neither in the rhizomes of healthy plants or in the fungal extracts.

Now and from healthy (non infected) rhizomes of the *Musa* selected hybrid cultivar SH-3481, which has been reported[10] to be resistant to the fungi *Fusarium oxysporum* f.sp. *cubense* races 1 and 4 and *Micosphaerella fijiensis,* we have isolated a new acenaphtylene derivative **1** and two new dimeric phenylphenalenones **2** and **3** together with the know phytoalexines: 2-hydroxy-4-(4-hydroxy-phenyl)-phenalen-1-one[6,7] , 2-hydroxy-4-(4-methoxy-phenyl)-phenalen-1-one[6] , 2-hydroxy-9-phenyl-phenalen-1-one[7] , trans-2,3-dihydroxy-2,3-dihydro-9-phenyl-phenalen-1-one[7] , 2-hydroxy-9-(4-hydroxy-phenyl)-phenalen-1-one[8,9] , 2-hydroxy-9-(4-hydroxy-3-methoxy-phenyl)-phenalen-1-one[9] and 1-[1-hydroxy-3-(4-hydroxy-phenyl)-allyl]-6-hydroxy-7-methoxy-1,2,3,4-tetrahydro-naphtalen-2-ol[8] which are now present as constitutive natural antibiotics and so as phytoanticipins[11] in this resistant banana cultivar.



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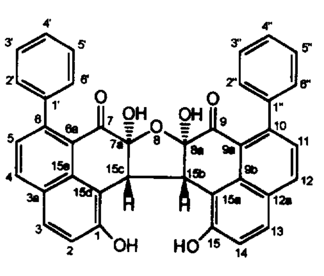

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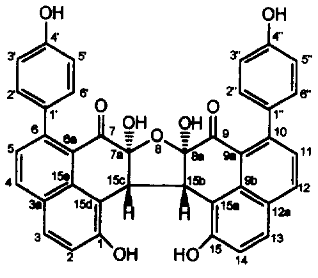

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The known phytoalexins were identified by comparison of their spectroscopic data with these of authentic samples. The isolation of this substances like phytoanticipins in this resistant hybrid cultivar, suggests that this type of phytoalexins play an important role as part of the resistant mechanisms to fungal diseases in this important alimentary resource botanic family.

Compound **1** was isolated as a colourless oil which did not show a band for the carbonyl group in the IR spectrum. The low resolution mass spectrum showed $[M]^+$ at m/z 262 ($C_{18}H_{14}O_2$ by HRMS). On the ^1H NMR spectrum signals were observed for an AMX, aromatic system like two doublets at δ7.43 (*J*=7.4 Hz) and δ7.78 (overlaped signal with a broad doublet) and a triplet at δ7.52; an AB aromatic system for two protons as two doublets centered at δ7.67 (*J*=8.4 Hz) and δ7.87 (*J*=8.4 Hz); a two protons broad doublet at δ7.78 coupled with a three protons multiplet signal (δ7.61) assignable to the phenol substituent and an AB system for two protons as two doublets centered at δ5.64 and 5.54 (*J*=5.6 Hz). This latter coupling locating the hydroxyl groups on adjacent carbon atoms and the value of the *J* constant, indicating a relative cis disposition for the hydroxy groups. The ^{13}C NMR showed signals for eighteen carbon atoms of which those resonating at δ74.32 and 72.52 are assignable to carbons bearing oxygen atoms and confirmed the absence of carbonyl group. The structure of **1** was fixed by combined HMQC and HMBC experiments (see Table 1).

All this data are in accordance with the structure of 3-phenyl-1,2-dihydroacenaphtylen-1,2-diol **1** for this new compound. At the best of our knowledge, this is the first acenaphtylene derivative yet isolated from a natural source.

Proton	**Three-bond correlation**	**Two-bond correlation**
H-2	C-8b. C-3	-----
H-4	C-1', C-2a	-----
H-6	C-8a, C-5, C-8	-----
H-5	C-8a, C-6	-----
H-7	C-5a, C-8a	-----
H-8	-----	C-8a
H-2', H-6'	C-4'	-----

Table 1.- Three and two-bond correlations for compound **1** in the HMBC experiment.

The new phenylphenalenone dimeric compound **2** was isolated as a yellow solid and its structure was established as follows. Low resolution mass spectrum showed the higher ion at m/z 287 ($C_{19}H_{11}O_3$ by EIHRMS). The IR spectrum had bands for phenol (3600 cm^{-1}), carbonyl (1708 cm^{-1}) and ether (1224 cm^{-1}) groups. A combination of ^1H NMR and ^1H-^1H COSY experiments showed the presence for the following systems: two aromatic AB systems, one of them as two protons doublets each centered at δ 7.01 and 7.68 (*J*=9.0 Hz) and other one as two protons doublet each, centered at δ 7.41 and 8.02 (*J*=8.2 Hz); a complex system at δ 7.45 and 7.52 integrating fot five aromatic protons; an aliphatic proton singlet at δ 4.14 and finally a broad singlet at δ 5.57 interchangeable with deuterium oxide.

The ^{13}C NMR shows the presence of only nineteen carbon atoms in the molecule of which ten of them are methine groups (that resonating at δ 32.50 being assignable to the aliphatic methine carbon which shows coupling on the HMQC experiment with the aliphatic proton H-15c at δ 4.14), nine quaternary carbon atoms one of them is carbonylic (δ 190.25) and a doubly quaternary oxygenated carbon, typical of hemiacetalic group at δ 92.17.

The assignment of all the hydrogen and carbon atoms in the molecule was made by combined HMQC and HMBC experiments (see Table 2). In this later the more significative datum was the observed two-bond correlation between, apparently, H-15c and C-15c which is only compatible with a totally symmetric dimer such as **2** in which the chemical shifts of the protons and carbons in the NMR spectrum would be totally superpose for both parts of the molecule corresponding in fact the above two-band correlation to that of H-15c and H-15b with C-15b and C-15c respectively and viceverse.

Proton	Three-bond correlation	Two-bond correlation
H-15c	C-15a	C-15c
7a-OH	C-15c, C-7	C-7a
H-2	C-15d, C-3a, C-1	-----
H-5	C-3a, C-6a	-----
H-3	C-15e, C-4, C-1	-----
H-4	C-15e, C-3, C-6	-----
H-3', H-5'	C1'	C-4'
H-2', H-6'	C-4'	-----

Table 2.- Three and two-bond correlations for compound **2** in the HMBC experiment.

The FAB MS confirmed the dimeric nature of the molecule, showing the higher ion fragments at m/z 591 [M^{+}-1] and m/z 575 [M^{+}-16]. From the chemical shift of the doublet assignable to H-4 and H-12 (δ 8.02) the lateral phenyl group must be linked to the carbon C-6[5,7].

Minimal energy calculation on the molecule **2** using the PCMODEL Program (Figure 1), indicated a 90 degrees dihedral angle between the aliphatic protons H-15b and H-15c, explaining the observed singlet signal for this protons on the ^{1}H NMR spectrum and establishing the relative configuration on the carbon atoms C-7a,C-7b,C-15b and C-15c as that show, with the molecule adopting a semihelicoidal conformation which may account for the little observed optical activity for this compound. All this data are in agreement with the proposed structure of 1,7a,8a,15-tetrahydroxy-6,10-diphenyl-7a,8a,15b,15c-tetrahydro-7H,9H-diphenalen-[1,2-b:1,2-d]-furane-7,9-dione for this new compound **2**.

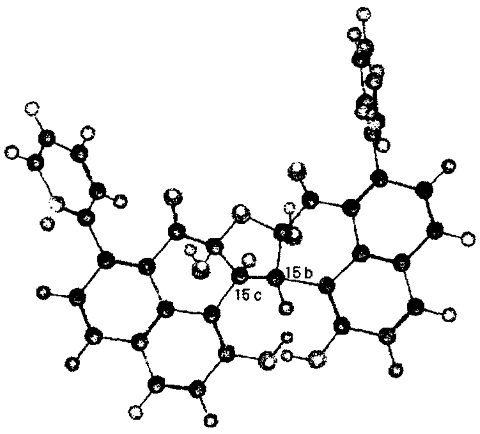

FIGURE 1.- Minimized structure of compound **2** as plotted by PCMODEL Program.

Product **3** was isolated like a yellow oil. The low resolution mass spectrum showed the higer ion at m/z 304 ($C_{19}H_{12}O_4$ by HRMS). The IR spectrum showed bands for phenol (3610 cm^{-1}), unsaturated carbonyl (1683 cm^{-1}) and ether (1255 cm^{-1}) groups. In the ^{1}H MNR the following systems were observed: two AB aromatic systems one of them as two doublets for two protons each centered at δ 8.08 and 7.39 (*J*=8.4 Hz) and the other as two doublets for two protons each centered at δ 6.96 and 7.76 (*J*=9.0 Hz); a AA'BB' system at δ 7.29 and 6.94 (*J*= 8.2 Hz); a proton singlet at δ 4.15 and finally a broad singlet interchangeable with deuterium oxide. This data are in accordance with the structure **3** for this new compound, the only difference with **2** being the presence of aditional phenolic groups on the phenyl substituents.

The ^{13}C NMR showed signals for nineteen carbon atoms one of those resonating at δ 94.00 is in accordance with a hemiacetalic group like in compound **2**.

Proton	**Three-bond correlation**	**Two-bond correlation**
H-15c	C-15a	C-15b
H-2	C-15d, C-3a	C-1
H-3	C-4, C-15e, C-1	-----
H-4	C-3, C-6	C-3a
H-5	C-6a, C-3a	C-4
H-3', H-5'	C-1'	C-4'
H-2', H-6'	C-4', C-6	C-1'

Table 3.- Three and two-bond correlations for compound **3** in the HMBC experiment.

The assignment of all the hydrogen and carbon atoms in the molecule was made by combined HMQC and HMBC (see Table 3) experiments, this later showing again the apparent self two bond correlation between H-15c and C-15c.

EXPERIMENTAL

General.- ^{1}H and ^{13}C and bidimensional spectra were recorded on Bruker AMX400 and WP200SY spectrometers, IR spectra were taken on a Bruker IFS 28/55 (FTIR) spectrometer, UV spectra were taken on a JASCO V-560 instrument and $[\alpha]_D$ on a PERKIN ELMER Mod 141 polarimeter. High resolution mass spectra were run on a VG-MICROMASS ZAB-2F at 70 eV. Low resolution mass spectra were run on a HEWLETT-PAKARD model 5995 and VG-MICROMASS ZAB-2F.

Extraction and isolation of Products.- Freshly collected rhizomes of healthy (uninfected) plant (11.0 Kg) of selected hybrid SH-3481 were immediately chopped and pressed to eliminate the water which was collected and immediately extracted with $CHCl_3$. The residual material was kept under maceration with EtOH 96% (20.0l) at room temperature for five days. The filtered EtOH extract was evaporated in a rotary evaporator and mixed with the above chloroformic extract, giving a material (13.5g) which was subjected to silica gel column using successively mixture of n-Hexane/AcOEt (9:1), n-Hexane/AcOEt (1:1), AcOEt and MeOH.

The n-Hexane/AcOEt (9:1) washing containing phytoanticipines was evaporated to dryness to yield a semicrude material (4.0g) which was subjected to SEPHADEX LH-20 column using a mixture of n-Hexane/$CHCl_3$/MeOH (2:1:1) as eluent to give: 2-hydroxy-4-(4-hydroxy-phenyl)-phenalen-1-one (2.0 mg), 2-hydroxy-4-(4-methoxy-phenyl)-phenalen-1-one (0.8 mg), 2-hydroxy-9-phenyl-phenalen-1-one (20.0 mg), trans-2,3-dihydroxy-2,3-dihydro-9-phenyl-phenalen-1-one (5.0 mg), 2-hydroxy-9-(4-hydroxy-phenyl)-phenalen-1-one (3.0 mg), 2-hydroxy-9-(4-hydroxy-3-methoxy-phenyl)-phenalen-1-one (2.0 mg) and 1-[1-hydroxy-3-(4-hydroxy-phenyl)-allyl]-6-hydroxy-7-methoxy-1,2,3,4-tetrahydro-naphtalen-2-ol (3.5 mg). The fractions which contained compounds **1-3** were purified by chromatography column on silica gel and then by preparative TLC.

3-Phenyl-1,2-dihydroacenaphtylen-1,2-diol (1).- (2.5 mg) was isolated as an amorphous solid; $[M]^+$ at m/z 262.098507 (calc. for $C_{18}H_{14}O_2$, 262.099380); IR ν_{max} ($CHCl_3$) cm^{-1}: 3027, 1540, 1261, 1096, 1015, 808; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.54 (1H, d, *J*=5.6 Hz, H-2), 5.64 (1H, d, *J*=5.6 Hz, H-1), 7.43 (1H, d, *J*=7.4 Hz, H-8), 7.52 (1H, t, *J*=7.4 Hz, H-7), 7.61 (1H, m, H-4'), 7.67 (1H, d, *J*=8.4 Hz, H-4), 7.78 (4H, br d, *J*=7.0 Hz, H-3', H-2', H-5', H-6' and H-6), 7.87 (1H, d, *J*=8.4 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$)

δ: 72.59 (d, C-2), 74.32 (d, C-1), 121.39 (d, C-4'), 124.74 (d, C-6), 126.14 (d, C-5), 127.54 (d, C-8), 128.32 (d, C-7), 128.74 (d, C-2' and C-6'), 128.83 (s, C-8a), 128.91 (d, C-3' and C-5'), 129.96 (d, C-4), 130.37 (s, C-2a), 136.43 (s, C-8b), 138.40 (s, C-1'), 139 (s, C-5a), 142.81 (s, C-3); EIMS (rel. int.) m/z: 262 $[M]^+$ (78), 244 (41), 234 (20), 216 (59), 215 (100), 202 (30), 189 (14), 128 (10), 107 (15), 94 (12), 63 (7), 51 (27).

1, 7a, 8a, 15-Tetrahydroxy-6, 10-diphenyl-7a, 8a, 15b, 15c-tetrahydro, 7H, 9H-diphenalen-[1,2-b:1,2-d]-furane-7,9-dione (2).- (9.0 mg) isolated as an amorphous yellow solid. M.P.: 82-84 °C; $[M^+-305]$ at m/z 287.069536 (calc. for $C_{19}H_{11}O_3$, 287.070819); $[\alpha]_D^{25}$: +3.60° ($CHCl_3$; c 0.39); UV λ_{max}(EtOH) nm: 249, 252, 282, 360; IR ν_{max}($CHCl_3$) cm^{-1}: 3600, 3450, 1708, 1558, 1224, 1218,1181,799; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.14 (2H, s, H-15c and H-15b), 5.57 (2H, s, -OH), 7.01 (2H, d, *J*=9.0 Hz, H-2 and H-14), 7.41 (2H, d, *J*=8.2 Hz, H-5 and H-11), 7.45 (4H, m, H-2', H-6', H-2" and H-6"), 7.52 (6H, m, H-3', H-5', H-4', H-3", H-4" and H-5"), 7.68 (2H, d, *J*=9.0 Hz, H-3 and H-13), 8.02 (2H, d, *J*=8.2 Hz, H-4 and H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.50 (d, C-15c and C-15b), 92.17 (s, C-7a and C-8a), 107.81 (s, C-15d and C-15a), 119.32 (d, C-2 and C-14), 122.22 (s, C-6a and C-9a), 127.62 (d, C-5 and C-11), 128.24 (d, C-2', C-4', C-6', C-2", C-4" and C-6"), 128.30 (s, C-3a and C-12a), 128.40 (d, C-3', C-5', C-3" and C-5"), 128.90 (d, C-3 and C-13), 132.91 (s, C-15e and C-9b), 134.50 (d, C-4 and C-12), 141.38 (s, C-1' and C-1"), 145.91 (s, C-6 and C-10), 152.23 (s, C-1 and C-15), 190.26 (s, C-7 and C-9); FAB-MS m/z 591 $[M^+-1]$, 575 $[M^+-16]$; EIMS (rel. int.) m/z: 287$[M^+-305]$ (57), 271 (7), 258 (4), 242 (3), 202 (10), 149 (11).

1,7a,8a, 15-Tetrahydroxy-6, 10-di-(4-hydroxyphenyl)- 7a, 8a, 15b, 15c-tetrahydro-7H, 9H-diphenalen-[1,2-b:1,2-d]-furane-7,9-dione (3).- (5.0 mg) isolated as an yellow oil. $[M^+-321]$ at m/z 303.066047 (calc. for $C_{19}H_{11}O_4$, 303.065734); $[\alpha]_D^{25}$: 10.0° ($CHCl_3$; c 0.20); UV λ_{max} (EtOH) nm: 294, 292, 264, 247, 246, 243, 242; IR ν_{max} (C_2H_6CO) cm^{-1}: 3609, 2254, 1745, 1255, 1265, 1034, 902; ^1H NMR (400 MHz, C_2H_6CO) δ: 4.15 (2H, s, H-15c and H-15b), 6.94 (4H, d, *J*=8.2Hz, H-2', H-6', H-2" and H-6"), 6.96 (2H, d, *J*=9.0Hz, H-2 and H-14), 7.29 (4H, d, *J*=8.2Hz, H-3', H-5', H-3" and H-5"), 7.40 (2H, d, *J*=8.4Hz, H-5 and H-11), 7.76 (2H, d, *J*=9.0Hz, H-3 and H-13), 8.08 (2H, d, *J*=8.4Hz, H-4 and H-12); ^{13}C NMR (100MHz, C_2H_6CO) δ: 33.54(s, C-15c and C-15b), 94.00 (s, C-7a and C-8a), 109.04 (s, C-15d and C-15a), 115.51 (d, C-2', C-6', C-2" and C-6"), 115.51 (d, C-2 and C-14), 119.28 (d, C-5 and C-11), 124.11 (s, C-6a and C-9a), 128.58 (s, C-3a and C-12a), 129.28 (d, C-3 and C-13), 130.85 (d, C-3', C-5', C-3" and C-5"), 133.27 (d, C-4 and C-12), 133.63 (s, C-15e and C-9b), 145.00 (s, C-6 and C-10), 145.89 (s, C-1' and C-1"), 152.68 (s, C-1 and C-15), 157.83 (s, C-4' and C-4"), 190.96 (s, C-7 and C-9); EIMS (rel. int.) m/z: 303 $[M^+-320]$ (88), 287 (23), 284 (28), 282 (31), 269 (24), 247 (15), 221 (48), 220 (53), 213 (54), 203 (89), 202 (40), 189 (30), 91 (94), 77 (54).

Acknowledgements: This research has been partly subsidized by Grant AMB95-0428-C02-02 from Spanish Government. We are grateful to Dr. F. García from the semipublic enterprise CULTESA (Tenerife) for submmiting the plant material.

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(*Received in UK* 24 *March* 1997; *revised* 28 *April* 1997; *accepted* 1 *May* 1997)