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Intermediates With Biosynthetic Implications In *De Novo* Production Of Phenyl-Phenalenone-Type Phytoalexins By *Musa acuminata* Revised Structure Of Emenolone

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Abstract: Three new intermediates (**1** - **3**) in *de novo* biosynthetic pathway to phenyl-phenalenone-type phytoalexins have been isolated from rhizomes of *Musa acuminata* infected with the fungus *Fusarium oxysporum*. The structures of the new pre-phytoalexins were elucidated on the basis of spectroscopic evidences, chemical correlation and acid catalyzed biomimetic cyclization of **1** to emenolone (**4**), isolated from the same source and whose previously reported structure has been unambiguously corrected by X-ray diffraction analysis. The chemical shift for all of the hydrogen and carbon atoms in the substances were unambiguously established by mono- and bidimensional, homo- and heteronuclear NMR experiments (^{1}H NMR, ^{13}C NMR, COSY, ROESY, HMQC and HMBC).

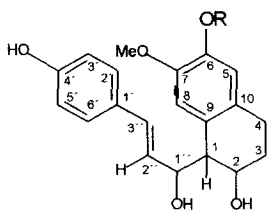
The phytoalexins are a group of natural products defined by their physiological rather than their structural features. These compounds are produced *de novo* by some plant organs when provoked by physical, microbiological, or chemical agents. The phytoalexins represent a structurally diverse group of compounds[1-3]. 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[1]. Emphasis has been rightly placed on the molecular biology and genetic of interactions between plants and bacteria or viruses. 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].

Recently, we have described the isolation, characterisation and synthesis[5-7] of the first examples of a new series of phytoalexins, structurally based on 9-phenyl-phenalene and 4-phenyl-phenalene nucleus, from *Musa acuminata* (AAA) Grand Nain, elicited with kanamycin, or infected for both of the fungi *Mycosfaerella*

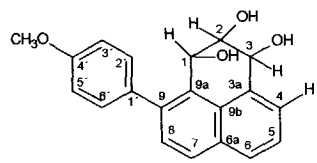
fijensis (causal agent of Black Sigatoka disease in banana plants) and *Fusarium oxysporum f. sp. cubense race 4* which causes Panama's disease in banana plants.

Several compounds with a phenalenone nucleus have been reported as normal metabolites in plants and microorganisms[8]. The plant phenalenones isolated until now possess typically a side phenyl ring on C-9 and have been found in species of the *Haemodoraceae* plant Family, in the genera *Haemodorum, lachnanthes, Xiphidium, Wachendorfia and Anigozanthus*, while microbial phenalenones, which do not have side phenyl ring, have been reported in *Hypomycetous (genera Penicillium, Fussicoccum, Giesmaniella and Verticilium)* and *Dyscomicetous* fungi. Nevertheless, no 4-phenylphenalenones had before been described either as natural or synthetic substances and none of this type of compounds has never been described before as phytoalexins i.e. produced *de novo* as a defensive response against pathogenic agents, as is the case for *Musa acuminata*.

Now and from rhizomes of *Musa acuminata* Grand Nain, infected with *Fusarium oxysporum*, we have isolated three intermediates (**1** - **3**) with biosynthetic implications in *de novo* production of phenyl-phenalenone-type phytoalexins by *Musa acuminata*.



1 R= Me
2 R= H



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The substances were isolated together, and separated out of its mixture with the phytoalexins from eighty Kg of rhizomes of banana plants which showed clear symptoms of Panama's disease, treated as previously reported[6-7], and their structures established as follows.

The less polar compound **1** had absorption maxima at 274, 351 and 382 nm in the UV spectrum and bands for phenols (3389 cm^{-1}) alcoholic (3500 cm^{-1}) and ether (1234 cm^{-1}), but not for carbonylic group, in the IR spectrum. Mass spectrometry showed an $[M - H_2O]^+$ ion at m/z 338.15114 (corresponding to $C_{21}H_{22}O_4$ by HRMS). All the hydrogen and carbon atoms in the molecule (Table 1) were discerned by mono- and bidimensional, homo- and heteronuclear NMR experiments (^1H NMR, ^{13}C NMR, COSY, ROESY, HMQC and HMBC). The ^{13}C NMR showed, in the DEPT experiment, the presence of signals for: eleven CH groups, two of them (δ 72.4 and δ 68.8) bearing oxygen atom, six quaternary carbon atoms, three of which (δ 147.7, δ 148.1, and δ 155.9) also bear oxygen atom and two CH_2 groups. Conjunct analysis of this spectrum and of the mass spectrum accounts for a molecular formula $C_{21}H_{24}O_5$ and for the existence in the molecule of ten unsaturation degrees, of which eight are assignable to two aromatic rings. In the ^1H NMR spectrum, the COSY experiment shows the presence of an aromatic AA'BB' spin system as two two-proton doublets at δ 6.81 and δ 7.28 (J=8.6 Hz) that demonstrate the p-disubstituted nature of one of the aromatics rings. In the HMBC experiment (Table 1), a three-bond correlation can be observed between a broad singlet signal at δ

5.35 (interchangeable with deuterium oxide) and the AA′ (δ 6.81) part of the above AA′BB′ system ,that places the phenol group on this aromatic ring. Two additional no coupled aromatic-proton signals at δ 6.61 and δ 6.77, indicate its relative *para-* disposition each to other and demonstrate the tetrasubstituted nature of the second aromatic ring. Two of the substituents on this late aromatic ring are methoxy groups as indicated by the presence of two three-proton singlet signals at δ 3.85 and δ 3.88 in the ^{1}H NMR spectrum. In this same spectrum , signals for an additional double bond can be observed as an ABX system with the part A as a one-proton double double-doublet signal at δ 6.22 (J= 10.3, 3.8, 2.3), the part B as a one-proton double-triplet signal at δ 5.90 (J= 10.3, 2.3) and the part X as a one-proton broad singlet signal at δ 5.14. The downfield of this latter proton indicates it is geminal to an hydroxy group, which was confirmed by the chemical shift of the

Table 1. NMR Spectral Data of **1**

			HMBC
C / H	H δ (J in Hz)	C δ	C
1	3.49 (bs)	37.3 (d)	2′′, 3, 8, 10
2	4.25 (bs)	68.7 (d)	1′′, 4, 9
3	1.92, 2.13 (d oct., 2.6, 3.8)	26.4 (t)	1, 10
4	2.71, 2.95 (d hex., 3.0, 3,8)	26.7 (t)	2, 5, 9
5	6.61 (s)	111.9 (d)	4, 7, 9
6	-	147.7 (s)	-
7	-	148.0 (s)	-
8	6.77 (s)	111.7 (d)	1, 6, 10
9	-	129.9 (s)	-
10	-	128.6 (s)	-
1′′	5.14 (bs)	72.4 (d)	3′′, 2, 9
2′′	5.90 (dt, 10.3, 2.3)	128.2 8d)	1, 1′
3′′	6.22 (d dd, 10.3, 3.8, 2.3)	129.3 (d)	1′′, 2′, 6′
1′	-	133.4 (s)	-
2′, 6′	7.28 (d, 8.6)	129.8 (d)	3′′, 4′
3′, 5′	6.81 (d, 8.6)	115.6 (d)	1′
4′	-	155.9 (s)	-
-OCH_3	3.85 (s)	56.3 (q)	6, 7
-OCH_3	3.88 (s)	56.3 (q)	6, 7
ArO<u>H</u>	5.35 (bs)	-	3′, 5′

[a] Data were recorded in $CDCl_3$ at 200 MHz. Carbon multiplicities were determined by DEPT experiments.

corresponding carbon atom, that appears as a doublet signal at ppm 72.4 in the heteronuclear HMQC experiment. In the HMBC experiment (Table 1) the observed strong three-bond connectivity between the proton at δ 6.22 in the above allylic system and the two carbon atoms at ppm 129.8 (which show one-bond correlation with the BB′ protons at δ 7. 28 of the aromatic AA′BB′system, in the HMQC experiment),

establishes the presence of the partial structure *p*-(OH)ϕ-CH=CH-CH(OH)- in the molecule. Because no more sp^2-hybridized carbon atoms are observed in the NMR spectra of **1**, the found ten unsaturation degrees , taken in conjunction with the tetrasubstituted nature of the second aromatic ring, established the existence of a dimethoxy-hydroxy-tetrahydronaphtalene residue in the molecule.

The high field region of the ^1H NMR spectrum shows, in the COSY experiment, the existence of an A_2M_2X spin system with the part A_2 (assignable to the 2 x H-4 protons) as two one-proton double-hexuplet signals at δ 2. 71 and δ 2. 95 (J= 3.0 and 3.8 respectively); the part M_2 (assignable to the 2 x H-3 protons) as two one-proton double-octuplet signals at δ 1.92 and δ 2.13 (J= 2.6 and 3.8 respectively) and the part X as a broad singlet signal at δ 4.25. The low chemical shift of this late proton signal indicates it is geminal to the second hydroxy alcoholic group in the molecule, which is in accordance with the observed chemical shift for the corresponding carbon atom (HMQC experiment) , which appears as a doublet signal at δ 68.7 in the ^{13}C NMR spectrum. Finally, a one-proton broad singlet signal at δ 3.49 (assignable to H-1), that showed in the COSY experiment, mixed coupling with the X part (H-2) of the above A_2M_2X system and with the X part (H-1′′) of the allylic ABX system, established the junction between the two differentiated parts of **1**. This assert and the substitution pattern on the tetrahydronaphtalenic moiety of the molecule were confirmed by the observed three-bond correlations between the H-2 proton and the C-1′′,C-4 and C-9 carbon atoms in the HMBC experiment (Table 1). These data are in accordance with **1** to be *1-[1-hydroxy-3-(4-hydroxyphenyl)-allyl]* - *6,7-dimethoxy-1,2,3,4-tetrahydronaphtalen-2-ol*.

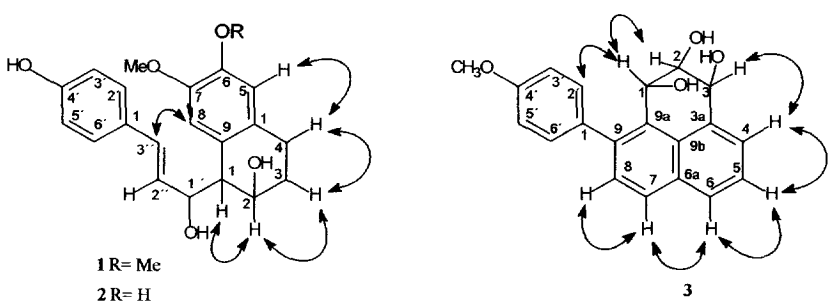


Figure 1

From the stereochemical point of view, the absolute configuration of **1** has not yet been established, because it was not possible to obtain a suitable crystal for X-ray diffraction analysis; but the very small coupling constant observed for the H-1 proton establishes a near 90° degrees dihedral angle between H-1 and H-1′′. The same is true for the dihedral angle between H-2′′and H-1′′, which appears as a broad singlet signal at δ 5.14. On the other hand, the existence of a nOe effect between H-3′′ and H-8 in the ROESY experiment (Figure 1) and the observed value for the coupling constant $J_{H2''-H3''}$=10.3 Hz , indicates a *trans*-stereochemistry for the allylic double bond. Of all the possible relative configurations on C-1, C-2 , C-1′′and

C-2'', that indicated in the Figure 2, obtained by molecular mechanic calculations using the PCMODEL program fitted the best the above stereochemical requirements, and the calculated coupling constants measured on it agree with the observed experimental values.

Chemical proof for the structure **1** followed (Scheme 1), when treatment of it with the Lewis acid, boron tribromide and then with DDQ (2,3- dichloro-5,6- dicyano-1,4- benzoquinone) a chemical agent that simulates very well the enzymatic dehydrogenation processes, gave as the major product a substance **4**, whose expected structure was unequivocally confirmed by X-ray diffraction analysis.

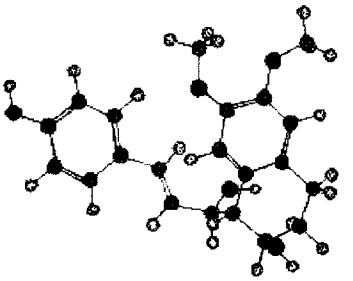
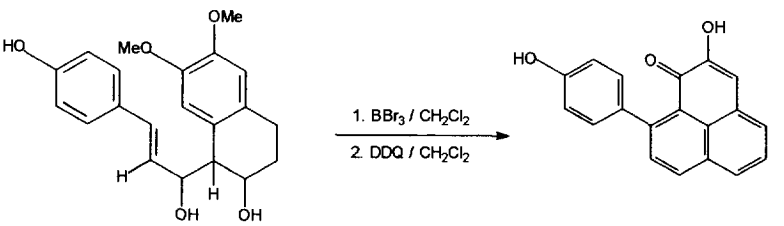


Figure 2

The molecular geometry of **4** with the atom-numbering is shown in Figure 3. The phenalene moiety is close to planar, the maximum deviation is 0.108(3)Å at C9. The greater deviations in the individual rings of this moiety are 0.033(3) Å at C9a , 0.040(3) Å at C9 and 0.022(4) Å at C5, the dihedral angles between this three rings are 4.18(9)°, 2.50(8)° and 4.12(8)°. There is not a relationship between the torsion angles and the bond lengths in ring A in the phenalene moiety, to smaller torsion angles correspond shorter bond lengths, but here is not the case, the bond length average is 1.43Å and the torsion angle average is 3°, therefore it should be more conjugated, which seems to have bond delocalization.



1 **4**

Scheme 1

The 01 at Cl and 02 at C2 are out of the plane for 0.137(2) Å and 0.020(3) Å respectively. The phenyl ring has normal geometry with the oxhydryl 03 almost coplanar, 0.059(2) Å with it. The dihedral angle between the phenyl ring and the phenalene group is 119.93(7)°, and the dihedral angles with the individual rings of this group are 118.72(9)°, 122.55(10)° and 120.37(9)°.

The view of the unit cell and packing, (Figure 4), shows that molecules are pack together by hydrogen bonds of type 0--H..... 0; the oxygen atoms 01, 02 and 03 are involved in two H-bonds. The geometrical parameters are as follow:

02..... 03= 2.853(3) Å ,	H(02)..... 03= 2.083(3) Å ,	02--H(02).....03=133.7(2)°
(1/2+x, -1/2-y, l+z) ;	03..... 01-2.853(5) Å	H(03).....01=1.990(3) Å ,
03--H(03)..... 01=142.6(2)° ,	(-1/2+x, -1/2-y, z)...	

Other contacts are consistent with van der Waals interactions.

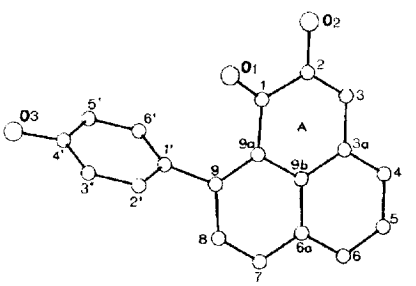


Figure 3. PLUTO[21] drawing of **4** with the atomic numbering

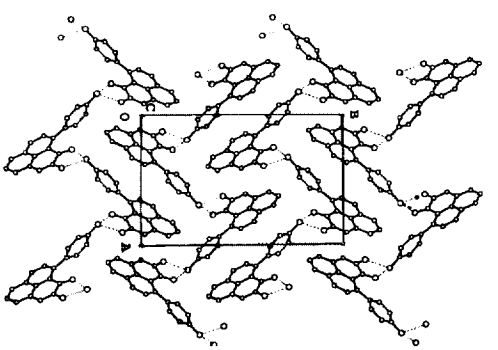


Figure 4

Compound **4**, Had identical spectroscopical characteristics as these of emenolone, a natural musanolone (9-phenyl-phenalenone-type phytoalexin) previously isolated for us[5] in very small quantity from *Musa acuminata* infected with the fungus *Micosphaerella fijensis* and for which the structure of 5-(4-hydroxyphenyl)phenalenone was suggested in basis of the comparison of its ^{1}H NMR with that of irenolone[5] [4-(4-hydroxyphenyl)phenalen-1-one] isolated from the same source; to the light of the above unequivocal evidences, the structure of emenolone must be corrected to **4**, a compound also isolated, and named hydroxyanigorufone[8], from *Anigozanthos rufus* (Haemodoraceae).

Compound **2** was characterised as *1-[1-hydroxy-3-(4-hydroxyphenyl)allyl]-6-hydroxy-7-methoxy-1,2,3,4-tetrahydronaphtalen-2-ol* as follows. It had absorption maxima at 272, 352, 378 and 382 nm in the UV spectrum and the IR spectrum was identical to that of **1**. Mass spectrometry showed a $[M - H_2O]^+$ ion at m / z 324.13616 (corresponding to $C_{20}H_{20}O_4$ by HRMS). The only two observed differences in the ^{1}H NMR

Table 2. NMR Spectral Data of **3**[a]

C / H	H δ (J in Hz)	C δ	HMBC C
1	4.99 (d, 2.7)	70.2 (d)	3, 9, 9b
2	3.81 (dd, 9.8, 2.7)	74.9 (d)	9a
3	5.25 (d, 9.8)	68.3 (d)	1,4 , 9b
3a	-	129.1 (s)	-
4	7.80 (dd, 8.2, 1.3)	122.9 (d)	3, 6, 9b
5	7.51 (t, 8.2)	127.4 (d)	3a, 6a
6	7.82 (dd, 8.2, 1.3)	126.0 (d)	4, 7, 9b
6a	-	132.7 (s)	-
7	7.88 (d, 8.3)	128.9 (d)	6, 9, 9b
8	7.46 (d, 8.3)	130.9 (d)	1′
9	-	149.5 (s)	-
9a	-	127.5 (s)	-
9b	-	129.6 (s)	-
1′	-	135.6 (s)	-
2′, 6′	7.49 (d, 8.6)	128.8 (d)	4′, 9
3′, 5′	6.96 (d, 8.6)	113.6 (d)	1′
4′	-	159.1 (s)	-
-OCH_3	3.84 (s)	55.3 (q)	4′
-OH	2.79, 2.91, 3.23	-	

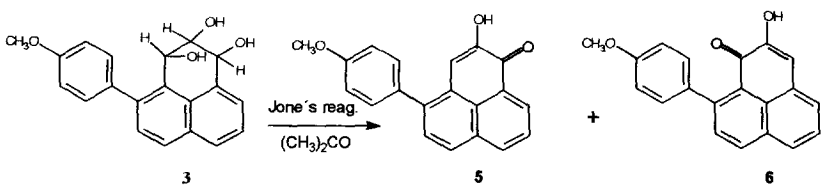
[a] Data were recorded in $CDCl_3$ at 200 MHz. Carbon multiplicities were determined by DEPT experiments.

spectrum of **2** as compared with that of **1** were the lack of the signal corresponding to one of the aromatic methoxy groups, indicating that **2** is a demethoxy-derivative of **1**, and a slightly lower chemical shift for the H-5 proton signal which now appears at δ 6. 67 as compared with δ 6. 61 for the same proton in the ^{1}H NMR

spectrum of **1**, which places the free phenol group in the catechol moiety of the molecule on C-6; this assert was confirmed by the observed nOe effect between the 7-$OC\underline{H}_3$ and the H-8 proton in the ROESY experiment.

The structure of the more polar compounds **3**, was established as being one of the possible diastereomers *of 1,2, 3-trihydroxy-1, 2-dihydro-9-(4-methoxyphenyl)phenalene*, as follows. It had absorption maxima at 268, 284, 352 and 388 nm in the UV spectrum and bands for alcoholic (3387 cm^{-1}) and ether (1245 cm^{-1}), but not for carbonylic group. Mass spectrometry showed the molecular ion $[M]^+$ at m / z 322.12038 (corresponding to a molecular formula $C_{20}H_{18}O_4$ by HRMS). All the hydrogen and carbon atoms in the molecule (Table 2) were unambiguously discerned by mono- and bidimensional, homo- and heteronuclear NMR experiments (^1H NMR, ^{13}C NMR, COSY, HMQC and HMBC).

The ^{13}C NMR spectrum showed, in the DEPT experiment, the existence of two well differentiated regions; an aromatic region (ppm 113.6 - ppm 159.1), that shows signals for nine CH groups and for seven quaternary carbon atoms, and an aliphatic zone (ppm 55.3 - ppm 74.9) with signals for three CH(OH) groups at ppm 68.3, ppm 70.2 and ppm 74.9 and for a $O\underline{C}H_3$ group at ppm 55.3. The corresponding aromatic region of the ^1H NMR spectrum shows, in the COSY experiment, three clear spin systems: a AA′BB′ system as two two-proton doublet signals at δ 6.96 and δ 7.49 (J= 8.6 Hz), assignable to a *p*-disubstituted aromatic ring, which bear the methoxy group as was demonstrated by the observed nOe effect between the three-proton singlet signal at δ 3.84 and the part AA′(δ 6.96) of the AA′BB′system in the ROESY experiment ; an AB system as two one-proton doublet signals at δ 7.88 and δ 7.46 (J= 8.3 Hz), whose part B (δ 7.46) shows in the HMBC experiment a strong three-bond connectivity with the carbon atom C-1′ (Characterized because it shows the same type of connectivity with the part AA′ of the cited AA′BB′system); and an AMX system with the part AM as two one-proton double doublet signals at δ 7.80 and δ 7.82 (J= 8.2, 1.3 Hz) and the part X as a one-proton triplet signal at δ 7.51 (J= 8.2 Hz) assignable to the H-4, H-6 and H-5 protons respectively. In the same ^1H NMR experiment, the aliphatic region constituted by the three C<u>H</u>(OH) groups, appears in the COSY experiment as other AMX system, with the part A as a one-proton doublet signal at δ 4.99 (J=2.7 Hz), the part M as a one-proton doublet signal at δ 5.25 (J= 9.8 Hz) and the part X as a one-proton double-doublet signal at δ 3.81 (J_1= 9.8 Hz, J_2= 2.7 Hz), that places the three hydroxy groups on adjacent carbon atoms. The three protons of the hydroxy groups appear in the ^1H NMR spectrum as three broad one-proton signals at δ: 2.79, 2.91 and 3.23, interchangeable with deuterium oxide.



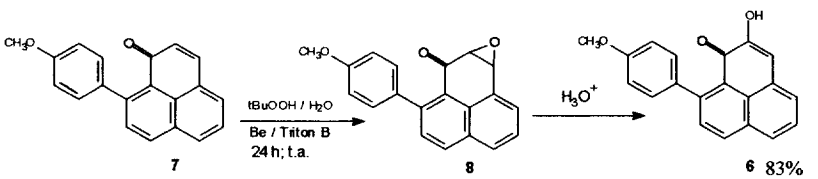
3 **5** **6**

Scheme 2

The assembling between the aliphatic and the aromatic parts of the molecule was deduced from the observed three-bond connectivities in the HMBC experiment (Table 2), of which the more important ones were these observed for the H-1 proton with the C-1′and C-9b carbon atoms (this latter carbon atom also

shows three-bond correlations with H-6 and H-7), and for the H-3 proton with the C-4 and C-9b carbon atoms.

The observed values for the coupling constants in the aliphatic AMX system: $J_{1,2}$= 2.7 Hz and $J_{2,3}$= 9.8 Hz indicate a relative *cis*- disposition between H-1 and H-2 and a *trans*- one for H-2 with H-3, which was confirmed by the observed strong nOe effect, in the ROESY experiment, between H-1 and H-2 and the lack of such effect between H-2 and H-3.



Scheme 3

Chemical confirmation for the structure **3** followed when oxidation of the substance with Jone's reagent in acetone (Scheme 2), gave the previously synthetized[6] compound **5** (major) and the compound **6** (minor),whose structure was confirmed by synthesis from **7**[6] as indicated in the Scheme 3.

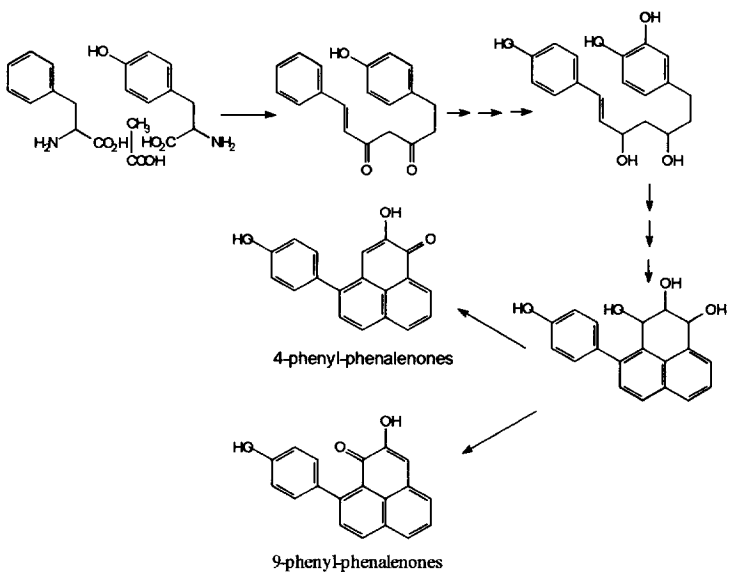
The relative yields of **5** and **6** in the above reaction are also in accordance with the proposed relative stereochemistry on C-1, C-2 and C-3.

It appears clear from the reactions in schemes 1 and 2 that all the three isolated substances are intermediates in *de novo* biosynthetic pathway to the phenalenone-type phytoalexins in *Musa acuminata*. The isolation of **3** and the reaction in Scheme 2 indicate that both series of phytoalexins: 9-phenyl-phenalen-1-ones and 4-phenyl-phenalen-1-ones come from a common 1,2,3-trihydroxy-2,3-dihydro-phenyl- phenalene intermediate (Scheme 4).

On the other hand, meanwhile the biosynthetic pathway to the natural phenalenone pigments found in some genus of *Penicillium* fungi (which do not bear phenyl lateral functionality), has been firmly established[9,10,11], the situation is not the same for the phenyl-phenalenone pigments isolated, as normal metabolites, from some plants of the *Haemodoraceae* Family[12,13]. Edwards[14,15] et al. have suggested from no conclusive incorporation experiments using 2-^{14}C-tyrosine (Thomas) and 1-^{14}C-phenylalanine and 1-^{13}C-phenylalanine (Edwards), that in this plant Family such pigments are formed through a biosynthetic route such as that indicated in the first step of the Scheme 4. It has been also suggested[16] that in this biosynthetic scheme, a biosynthetic equivalent of the Diels-Alder reaction participates in the cyclization step from the biarylheptanoid to the phenalenone nucleus.

In the case of the phenalenone-type phytoalexins from *Musa acuminata*, our findings indicate that although the intermediation of a biaryl-heptanoid appears to be clear and so comes, also in this case, in support of the biosynthetic scheme proposed by Thomas and Edwards with participation of one mole each of

phenylalanine, tyrosine and acetate; the intervention of a biosynthetic equivalent of the Diels-Ader reaction in the cyclization step to the phenalen moiety of the molecule is precluded. We are presently carrying out biosynthetic studies to try to clarify *de novo* formation of these substances by *Musa acuminata.*



Scheme 4

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX400 and WP200SY spectrometers. IR spectra were taken on a Perkin-Elmer 1600 (FTIR) spectrophotometer and UV spectra on a Perkin Elmer 550SE instrument. High resolution mass spectra were run on a VG-Micromass ZAB-2F at 70 eV.

Plant material

Rhizomes of Panama disease-infected plants and healthy plants were collected at the CITA experimental station in Tenerife.

Extraction and isolation

Freshly collected rhizomes of infected plants (80 kg) 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 l) at room temperature for 5 days. The filtered ethanolic extract was

evaporated in a rotavapor to 1/4 of its volume and repeatedly extracted with $CHCl_3$. The mixed chlorophorm extracts were evaporated to dryness to give 9.8 g of crude material, which was percolated through a silica gel column (300 g) using successively n-hexane, n-hexane-EtOAc (1:1), EtOAc and MeOH (1.5 l each).

The n-hexane-EtOAc (1:1) washing was evaporated to dryness to yield 5.4 g of semicrude material which was submitted to chromatographic separation on Sephadex LH-20 (500 g), using as eluent n-hexane: $CHCl_3$: MeOH (2:1:1). 57 Fractions of 40 ml were collected.

The rhizomes from healthy plants (80 kg) were treated in exactly the same way.

The above fractions 11-14 (0.59 g) were repeatedly chromatographed on silica gel (63-200 μ , Merck) using as eluent mixtures of n-hexane - EtOAc and the substances **1-3** were purified by preparative TLC (on precoated 0.25 mm silica gel plates from G. Schleicher & Schull). By increasing order of polarity: **1** (6.9 mg); **2** (2.0 mg) and **3** (5.2 mg).

Filtered out mycelia of *in vitro* cultured *Fusarium oxysporum. sp. cubense race 4* and the culture medium were both extracted with ethanol, and the combined alcoholic extracts were evaporated to dryness. This extract did not show the presence of phenyl-phenalenones on TLC.

***1-[1-Hydroxy-3-(4-hydroxyphenyl)allyl] - 6,7-dimethoxy-1,2,3,4-tetrahydronaphtalen-2-ol* (1).-** Colourless crystals; m.p. 82-84 °C (from Be/n-hexane); $[\alpha]_D^{25} = -51.2°$ (c, 0.112,$CHCl_3$); HREIMS: [M-18]$^+$ at m/z 338.15114 (calc. for $C_{21}H_{22}O_4$, 338.15181); IR ($CHCl_3$): ν_{max} / cm^{-1}: 3490, 3389, 3025, 2934, 2845, 1612, 1513, 1446, 1366, 1234, 1170, 1112, 1071, 1015, 952, 834, 754; UV(EtOH) λ_{max} / nm: 274, 351, 382; ^{1}H NMR and ^{13}C NMR (see Table 1); MS: m / z (rel. int. %): 338 [M-18]$^+$ (100), 31(4), 279(6), 224(19), 215 (14), 210(10), 187(37), 173(78), 151(68), 115(55), 109(33), 84(47), 55(28).

***Biomimetic cyclization of* 1.-** Compound **1** (4.5 mg) dissolved in dry CH_2Cl_2 (3.0 ml), were treated with 35 μl of a dissolution of BBr_3 in CH_2Cl_2. The stirred reaction mixture was kept at room temperature for 15 min, and then the reaction stopped by addition of 3.0 ml of methanol and evaporated to dryness in a rotatory evaporator. The crude material dissolved in CH_2Cl_2 (5 ml) was treated with DDQ (10 mg) , refluxed for 2 h and then stirred at room temperature overnight. Preparative TLC on silica gel, using n-hexane-AcOEt (3:2) as eluent, gave pure **4** (2.75 mg), which crystalized from benzene/n-hexane as red crystals.

***X-ray analysis of crystalline* 4.-** Crystal data: $C_{19}H_{12}O_3$, Mr=288.302, monoclinic, P21/a, Z=4, a=11.936(l), b=18.213(l), c=6.0096(2) Å, β=93.185(4)°; V=1304.4(1)Å^3, F(000)= 600.0, λ (KαCu)=1.5418Å, μ=7.622 (cm^{-1}, Dc=1.4680(mg / m^{-3}).

The crystal of title compound appears as red colour stout monoclinic prism. A prism fragment of 0.28 x 0.22 x 0.18 mm, was selected for data collection. Lattice parameters from least-squares adjustment to setting angles of 31 reflections with $10° < \theta < 40°$; 2287 reflections recorded on a Philips 1100 PW diffractometer, with monochromatyed KαCu radiation in the range $2° < \theta < 65°$ using $\omega / 2\theta$ scan mode, scan width 1.50, scan speed 0.06° seg^{-1}. with ranges in h, k , l : $-14 \le h \le 14$, $0 \le k \le 21$, $0 \le l \le 7$. The intensities of two standard reflections measured every 90 reflections through the data collection remained constant. 2287 reflections were measured and finally 2021 with $I>2\ \sigma$ (I) kept for structure determination. Intensities corrected for Lorenz and polarization effects, but not absorption correction was applied. The structure was solved by direct methods (MULTAN)[17], and Fourier difference maps. The structure refined by full-matrix

least-squares with isotropic and later anisotropic temperature factors. All H-atoms were located in difference maps, and they were include in the last cycles of refinement and refined with isotropic thermal parameters equal to their carrier atoms.

An empirical weighting scheme[18] was applied just not to give dependence in $< w\Delta^2 F >$ vs. <Fo> and < sin θ / λ >; the final R and Rw values are 7.8% and 10.1%. The final difference Fourier synthesis did not reveal any peak of density >0.36 eÅ^{-3}. The high R value is caused by the weakly diffracting crystal. All calculations were performed on the VAX 6410 computer using the XRAY76 System programs[19], and several local programs; the scattering atomic factors from International Tables of Crystallography[20].

1-[1-Hydroxy-3-(4-hydroxyphenyl)allyl]-6-hydroxy-7-methoxy-1,2,3,4-tetrahydronaphtalen-2-ol **(2).**-Colourless crystals; m.p. 112-115°C (from Be/n-hexane); $[\alpha]_D^{25}$ = -52.6° (c, 0.095,$CHCl_3$); HREIMS: $[M-18]^+$ at m/z 324.13602 (calc. for $C_{20}H_{20}O_4$, 324.13616); IR ($CHCl_3$): ν_{max} / cm^{-1}: 3490, 3382, 2936, 2845, 1612, 1514, 1450, 1356, 1259, 1170, 1115, 1032, 839, 754; UV(EtOH) λ_{max} / nm: 272, 352, 378, 382; ^{1}H NMR ($CDCl_3$) δ: 1.92 (1H, oct, J=2.6 Hz, H-3α), 2.13 (1H, oct, J= 3.8 Hz, H-3β), 2.72 (1H, hex,J= 3.0 Hz, H-4α), 2.93 (1H, hex, J= 3.8 Hz, H-4β), 3.47 (1H, bs, H-1), 3.89 (3H, s, -OC<u>H</u>$_3$), 4.25 (1H, q, J= 3.2 Hz, H-2), 5.15 (1H, bs, H-1´´), 5.37 (1H, bs, Ar-O<u>H</u>) , 5.52 (1H, bs, Ar-O<u>H</u>), 5.90 (1H, dt,J_1=10.3, J_2= 2,4 Hz; 6.2 Hz, H-2´´), 6.20 (1H, d dd J_1=10.3, J_2=3.8, J_3= 2.3 Hz, H-3´´), 6.62 (1H, s, H-8), 6.81 (2H, d, J= 8.6 Hz, H-3´and H-5´), 7.29 (2H, d, J= 8.6 Hz, H-2´and H-6´); MS: m/z (rel. int. %): 324 $[M-18]^+$ (57), 176(20), 173(100), 161(12), 137(44), 131(13), 78(23), 55(14).

1, 2, 3-Trihydroxy-1, 2-dihydro-9-(4- methoxyphenyl)-phenalene **(3)**.- Colourless crystals; m.p. 104-106 °C (from Be/n-hexane); $[\alpha]_D^{25}$ = -15.38° (c, 0.26,$CHCl_3$); HREIMS: $[M]^+$ at m/z 322.12038 (calc. for $C_{20}H_{18}O_4$, 322.12051); IR ($CHCl_3$): ν_{max} / cm^{-1}: 3387, 3004, 1608, 1515, 1498, 1245, 1178, 1085, 1025, 920, 824, 758; UV(EtOH) λ_{max} / nm: 268, 284, 352, 388; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) (see Table 2); ^{1}H NMR (C_6D_6) δ : 2.90 (1H, bs, -O<u>H</u>), 2.99 (1H, bs, -O<u>H</u>), 3.11 (1H, bs, -O<u>H</u>), 3.30 (3H, s, -OC<u>H</u>$_3$), 3.49 (1H, dd, J_1= 9.8 Hz, J_2= 2.8 Hz, H-2), 4.96 (1H, d, J= 2.8 Hz, H-1), 5.20 (1H, d, J= 9.8 Hz, H-3), 6.80 (2H, d, J= 8.8 Hz, H-3´and H-5´), 7.28 (1H, d, J= 8.7 Hz, H-8), 7.35 (2H, d, J= 8.6 Hz, H-2´and H-6´), 7.55 (3H, m H-4, H-5 and H-7), 7.85 (1H, dd, J_1= 7.8 Hz, J_2= 1.3 Hz, H-6); MS: m/z (rel. int. %): 322 $[M]^+$(24), 302(20), 287(28), 202(21), 188(100), 167(24), 112(15), 97(10), 83(33), 55(71).

Oxidation of **3**.- Compound **3** (5 mg), dissolved in acetone (4.0 ml), was treated with three drops of a 5% dissolution of H_2SO_4 in H_2O. After stirring of the reaction mixture for 15 min, the total transformation of the starting material was confirmed by TLC. 0.5 ml Of Jone´s reagent were added and the stirring was continued for 15 more min. The reaction mixture was neutralised with saturated solution of $NaHCO_3$ and extracted with 3 x 10 ml of $CHCl_3$. The chloroform extract was dried on sodium sulfate, filtered and evaporated to dryness in a rotatory evaporator. Preparative TLC on silica gel using Be: Acetone (4:1) as eluent gave pure **5** (3.5 mg) and **6** (0.85 mg).

2,3-Epoxi-9-(p-meyhoxyphenyl)phenalen-1-one **(8)**.- 9-(p-methoxyphenyl)phenalen-1-one (7) (100 mg), prepared from perinaphtenone (Aldrich) as previously reported[6], was dissolved in benzene (15 ml) and treated at 0°C and successively with t-BuOOH (70% in H_2O, 120 μl) and benzyltrimethylammonium hydroxide

(triton B) (120 µl). The stirred reaction mixture was kept at room temperature for 24 h, washed with water, and the benzene evaporated to dryness in a rotatory evaporator. Preparative TLC of the crude reaction mixture yielded **8** (98 mg).). IR ($CHCl_3$): ν_{max} / cm^{-1}: 3015, 2957, 1677, 1516, 1453, 1352, 1244, 1117, 1031, 817; ^1H NMR ($CDCl_3$) δ: 3.88 (3H, s, $-OCH_3$), 4.18 (1H, d, J= 4.2 Hz, H-2), 4.62 (1H, d, J= 4.2 Hz, H-3), 6.70 (2H, d, J= 8.4 Hz, H-3′ and H-5′), 7.37 (2H, d, J= 8.4 Hz, H-2′and H-6′), 7.50 (1H, t, J=7.8 Hz, H-5), 7.58 (1H, d, J= 8.3 Hz, H-8), 7.83 (1H, dd, J_1= 8.3 Hz, J_2= 1.3 Hz, H-4), 7.93 (1H, dd, J_1= 8.3 Hz, J_2= 1.3 Hz, H-6), 8.06 (1H, d, J= 8.3 Hz, H-7); MS: m/z (rel. int. %): 302 $[M]^+$(48), 274(72), 259(20), 231(43), 202(100), 176(16), 63(12).

2-Hydroxy-9-(p-methoxyphenyl)phenalen-1-one **(6)** .- Compound **8** (50 mg) dissolved in dichloromethane (10 ml) was treated with p-toluensulphonic acid (10 mg) and the reaction mixture stirred at room temperature for 1 h . Preparative TLC of the crude reaction mixture gave **6** (48 mg); Orange crystals (from Be / n-Hexane); m.p. 202-205°C; IR ($CHCl_3$): ν_{max} / cm^{-1}: 3356, 1614, 1606, 1515, 1495, 1405, 1288, 1247, 1178, 1067, 1028, 820, 748; ^1H NMR ($CDCl_3$) δ: 3.91 (1H, s, $-OCH_3$), 7.05 (2H, d, J= 8.4 Hz, H-3′and H-5′), 7.10 (1H, s, -OH), 7.15 (1H, s, H-3), 7.36 (2H, d, J= 8.4 Hz, H-2′and H-6′), 7.59 (1H, t, J= 7.8 Hz, H-5), 7.62 (1H, d, J= 8.3 Hz, H-8), 7.74 (1H, dd, J_1= 8.3 Hz, J_2= 1.3 Hz, H-4), 7.94 (1H, dd, J_1= 8.3 Hz, J_2= 1.3 Hz, H-6), 8.23 (1H, d, J= 8.3 Hz, H-7); MS: m/z (rel. int. %): 302 $[M]^+$(61), 301(100), 287(58), 271(67), 259(87), 213(57), 202(93), 187(21), 88(20).

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