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Phenylphenalenone Type Compounds from the Leaf Fibers of Abaca (*Musa textilis*)

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A series of phenylphenalenone type compounds, known to play a role as phytoalexins in plants of the Musaceae family, have been identified for the first time in the leaf fibers of abaca (*Musa textilis*). Among the phenylphenalenone type compounds identified, the structure of a novel compound, (1*R*)-2,3-dihydro-4,9-dihydroxy-8-methoxy-1-phenylphenalene, has also been described in abaca fibers. Its structure was elucidated by analysis of one- and two-dimensional NMR (correlation spectroscopy, heteronuclear single quantum correlation, and heteronuclear multiple bond correlation) spectroscopic data.

KEYWORDS: Abaca; *Musa textilis*; Musaceae; phenylphenalenones; phytoalexins; GC/MS; NMR

INTRODUCTION

A series of phytoalexins structurally based on a phenalenone skeleton have been described in plants of the Musaceae family, including *Musa* and *Ensete* species. These compounds have been found in banana plants (*Musa paradisiaca* and *Musa acuminata*) infected with *Mycosphaerella fijiensis* (causal agent of Black Sigatoka disease), a pathogenic fungus that attacks banana leaves, or infected with *Fusarium oxysporum*, a saprophytic pathogenic fungus that causes Panama disease in banana plants (*1*−*4*). Indeed, phenylphenalenone type phytoalexins were also isolated from anthracnose-infected banana fruits (*5*, *6*) and also from *M. acuminata* infected with the burrowing nematode *Radopholus similes* (*7*). Because of their intense red or orange color, phenylphenalenones are visually detectable in pigmented roots and rhizomes, which seem to be the richest source of these natural products. In addition to these parts, phenylphenalenone-type compounds have also been detected in other parts of the plant, such as infected leaves (*8*, *9*).

Abaca (*M. textilis*) is a banana-like plant of the *Musa* species grown mainly in the Philippines. Its fiber, also known as Manila hemp, is obtained from the leaf sheath and has been traditionally used for ropes and cordage. Indeed, it is an excellent raw material for the manufacturing of specialty papers. Its long fiber length, high strength, and fineness make it a superior material for the production of thin, lightweight papers of high porosity and excellent tear, burst, and tensile strengths (*10*, *11*). The abaca fibers used for papermaking are usually pale to white colors; however, intense red color parts are often distinguished. Because abaca, like other *Musa* species, suffers from similar diseases as banana (*M. acuminata* or *M. paradisiaca*) (*12*, *13*), it is possible that these red color parts might be due to the presence in the fiber of phenylphenalenone type or related compounds.

In this paper, we have analyzed the acetone extracts of two parts of the same batch of abaca leaf fibers with different coloration in order to get insight into their chemical differences. The identification and structural elucidation of phenylphenalenone type phytoalexins in abaca fibers are described here for the first time.

MATERIALS AND METHODS

Samples. The leaf fibers from abaca (*M. textilis*) were supplied by CELESA pulp mill (Tortosa, Spain). Two distinct parts of the fiber having different coloration, light and red colors, were separated manually. The air-dried fibers (around 50 g) were milled using a knife mill (Janke and Kunkel, Analysenmühle) and extracted with acetone in a Soxhlet apparatus for 8 h. The extracts were evaporated to dryness and redissolved in chloroform for chromatographic analysis. Two replicates were used for each sample, and all of them were subjected to gas chromatography/mass spectrometry (GC/MS) analyses.

Solid Phase Extraction (SPE) Fractionation. The lipid extracts were fractionated by a SPE procedure using aminopropyl phase cartridges (500 mg) from Waters (Division of Millipore). The dried chloroform extracts were taken up in a minimal volume (<0.5 mL) of hexane:chloroform (4:1) and loaded into the cartridge column previously conditioned with hexane (4 mL). The cartridge was loaded and eluted by gravity. The column was first eluted with 8 mL of hexane and subsequently with 6 mL of hexane:chloroform (5:1), then with 10 mL of chloroform, and finally with 10 mL of diethyl ether:acetic acid (98:2). Each isolated fraction was dried under nitrogen and analyzed by GC/MS.

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Thin-Layer Chromatography (TLC). For the isolation and purification of selected compounds, the acetone extracts were subjected to TLC. Preparative silica gel plates (Merck) were used for that purpose. The plates were loaded with the extracts and subsequently developed with a mixture of hexane:diethyl ether (80:20). The band at R_f 0.16 was scraped off the plate and extracted with chloroform, and the extracts were subsequently dried under a stream of nitrogen. The purified compound was subsequently analyzed by NMR.

GC/MS. The GC/MS analyses were performed on a Varian Saturn 2000 (Varian, Walnut Creek, CA) with an ion trap detector, equipped with a fused silica capillary column (DB-5HT, J&W; 12 m × 0.25 mm i.d., 0.1 μm film thickness). The oven was heated from 120 (1 min) to 380 °C at 10 °C/min and held for 5 min. The transfer line was kept at 300 °C. The injector was temperature programmed from 120 (0.1 min) to 380 °C at a rate of 200 °C/min and held until the end of the analysis. Helium was used as the carrier gas at a rate of 2 mL/min. Trimethylsilyl-diazomethane methylation and bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation, in the presence of pyridine, and acetylation with acetic anhydride in the presence of pyridine were used to produce the appropriate derivatives, when required. Compounds were identified by comparing their mass spectra with those in the Wiley and NIST computer libraries, with those reported in the literature, and when possible by comparison with authentic standards. Quantitation was performed by peak area using appropriate standards as described previously (*14*).

NMR. The spectra of the samples, dissolved in deuterated chloroform (ca. 10 mM), were recorded on a Bruker AVANCE 500 spectrometer equipped with a triple resonance ^{1}H, ^{13}C, and ^{15}N probe with a gradient in the *Z* axis, at a temperature of 300 °K. Chemical shifts are in ppm with respect to the 0 ppm point of the manufacturer's indirect referencing method. The assignments of the signals were performed according to the standard correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) sequences provided by Bruker.

COSY and NOESY experiments were performed with 16 and 48 scans, respectively, with 256 increments in the indirect dimension and with 2048 points in the acquisition dimension. The spectral widths were 9 ppm in both dimensions. The HSQC experiment (16 scans) also used 256 increments in the indirect dimension with 2048 points in the acquisition dimension. The spectral width was 150 ppm in the indirect dimension and 9 ppm in acquisition. The HMBC experiment (32 scans) also used 256 increments in the indirect dimension with 2048 points in the acquisition dimension. The spectral width was 180 ppm in the indirect dimension and 9 ppm in acquisition.

RESULTS AND DISCUSSION

A comparison of the acetone extracts from the two different parts of the abaca fibers revealed noticeable differences between them. The total acetone extract from the light color parts of the abaca fibers accounted for 0.22% of the total fiber weight, while the acetone extract from the dark color fibers accounted for 0.36%. Indeed, the acetone extracts from the dark color fibers presented a characteristic and intense red color. Therefore, it seems clear that there is a significant difference in the content and composition of the extractable material in both parts of the fibers and that the compounds present in the latter extract might be responsible for the coloration of the fibers.

The acetone extracts were subsequently analyzed by GC/MS. **Figure 1** shows the chromatograms of the underivatized extracts from the two different parts of the abaca fibers. The GC/MS analysis of both parts also revealed a different pattern of compounds present in the extracts. The extracts of the light color part of the abaca fibers (**Figure 1A**) were constituted mainly by fatty acids such as palmitic acid (C_{16}), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), stearic acid (C_{18}), and sterols such as campesterol, stigmasterol, and sitosterol, steroid ketones such as cycloartenone, 24-methylenecycloartanone, stigmasta-3,5-dien-7-one, stigmast-4-en-3-one, and stigmastane-3,6-dione, and a series of *p*-hydroxycinnamic acids (*p*-coumaric and ferulic acids) esterified to long chain alcohols and ω-hydroxyfatty acids and sterol esters. The complete characterization, including lipid and lignin composition, of the abaca fibers has already been published elsewhere (*14*, *15*).

By contrast, the chromatogram of the extracts of the dark color parts of the fibers (**Figure 1B**) shows, besides the compounds also present in the light color fiber, a new series of peaks (I−VII) that were not present in the light color fibers and which seem responsible for the dark coloration of the fiber and the acetone extract. Among these compounds, the chromatogram was clearly dominated by compound **V**, which is by far the most abundant compound in the extract. Most of these compounds were identified by mass spectrometry as being phenylphenalenone type compounds (**Figure 2**). These compounds corresponded to the known compounds anigorufone (**I**), 2-hydroxy-4-phenylphenalen-1-one (**II**), methoxyanigorufone (**III**), 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one (**IV**), 4′-methoxyirenolone (**VI**), and 2-methoxy-9-(4′-methoxyphenyl)-phenalen-1-one (**VII**) and were identified according to the mass spectra published in the literature (*5*, *16*). All of these compounds have been detected previously in other *Musa* species, although this is the first time that they have been reported in abaca. However, the mass spectrum of compound **V** (**Figure 3**), which gives a molecular ion at *m*/*z* 306 that is also the base peak and important fragments at *m*/*z* 273, 229, 227, 211, and 197, does not correspond to any known compound. Appropriate derivatization to produce the acetyl or the TMS derivatives indicates the presence of two hydroxyl groups in its structure, but no further structural data could be obtained from the low-resolution MS.

Compound **V** was therefore further purified and concentrated by TLC and the structure elucidated using HR-MS as well as ^{1}H, ^{13}C, and two-dimensional NMR (COSY, HMBC, and HMQC) techniques. Compound **V** was obtained as an intense red solid (optical rotation −0.073, c 0.1, $ClCH_3$). A molecular formula of $C_{20}H_{18}O_3$ (*m*/*z* obs. 306.125590, calcd 306.125595) was assigned using HR-MS. The ^{1}H NMR spectrum (**Table 1**) of **V** displayed signals corresponding to two ortho-coupled aromatic protons (δ 6.89, d, J = 8.5 Hz; δ 7.44, d, J = 8.5 Hz), five aromatic protons of monosubstituted phenyl ring (δ 6.90, dd, J = 7.0, 1.0 Hz; δ 7.08, ddd, J = 7.0, 7.0, 1.0 Hz; and δ 7.13, dd, J = 7.0, 7.0 Hz), and one aromatic proton of a pentasubstituted phenyl ring (δ 6.98). The COSY spectrum indicated that the signals located at δ 2.12 (dddd J = 13.0, 13.0, 4.0, 4.0), δ 2.23 (dddd J = 13.0, 4.0, 2.5, 2.5), δ 2.44 (ddd, J = 16.0, 13.0, 4.0), and δ 2.84 (ddd, J = 16.0, 4.0, 2.5) corresponded to two vicinal methylenes and one of them is coupled directly bound to a benzylic methane, whose signal is located at δ 4.74 (dd, J = 4.0, 2.5 Hz). The presence of a methoxyl group (δ 3.90) at the quaternary carbon C-8 (δ 145.57) was established from the analysis of the HMBC spectrum. Furthermore, the presence of two hydroxyl hydrogens (located at δ 5.80 s and δ 4.65 br s) was established by the lack of cross-peaks for these signals in the HSQC spectrum. The connectivity of these fragments was made by the analysis of the HMBC data, establishing the characteristic phenylphenalenone type structure. The analysis started with two J^2 and J^3 correlations between each one of ortho-coupled aromatic protons, H-5 and H-6, with the phenolic quaternary carbon (C-4, located at δ 148.21). The phenolic hydrogen at δ 5.80 showed J^2 cross-peaks with the adjacent C-9 (located at δ 142.24) and J^3 with the C-8 (located at δ145.57). Additionally, the signal for H-7 (δ 6.98) exhibited

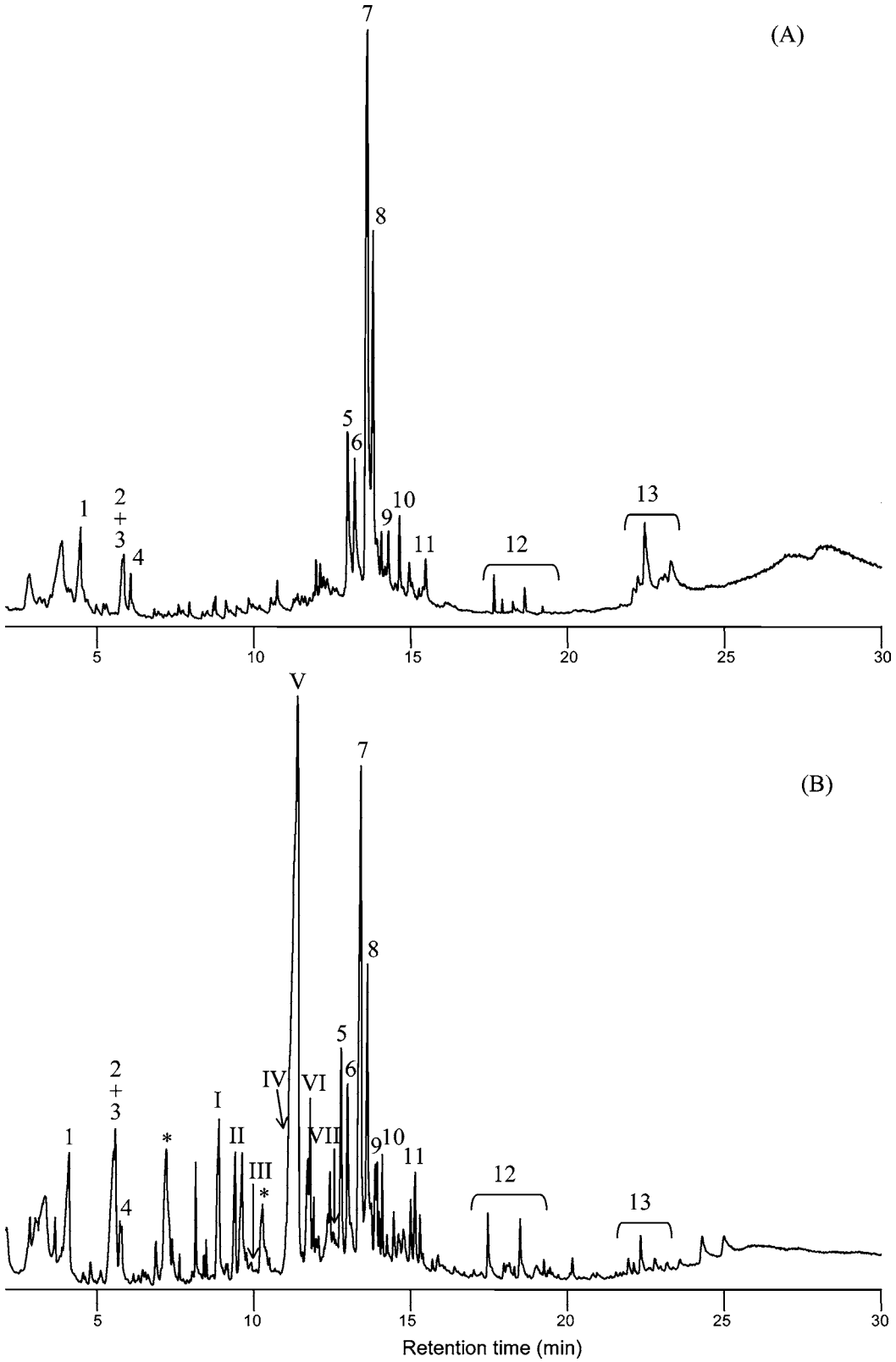

Figure 1. GC/MS of the underivatized extracts isolated from the two different parts of the abaca (*M. textilis*) fibers: (**A**) light color fibers and (**B**) dark color fibers. Labeling for compounds: 1, palmitic acid; 2, linoleic acid; 3, oleic acid; 4, stearic acid; 5, campesterol; 6, stigmasterol; 7, sitosterol; 8, cycloartenone; 9, stigmasta-3,5-dien-7-one; 10, stigmast-4-en-3-one; 11, stigmastane-3,6-dione; 12, *p*-hydroxycinnamic acid esters; and 13, sterol esters. I–VII, phenylphenalenone type compounds referred to in the text and shown in **Figure 2**.

a J^3 correlation with the quaternary carbon at C-9. The presence of naphthalene skeleton was evident by the presence of J^3 cross-peaks assigned to H-6 (δ 7.44)/H-7 (δ 6.98) with C-9b (δ 127.37) and H-5 (δ 6.89) with C-6a (δ 123.71). Furthermore, the HMBC correlations of the benzylic methine assigned to H-1 (δ 4.74) with the quaternary carbon C-9a (δ 117.64), as well as the J^2 and J^3 cross-peaks of the benzylic methylene H-3a,3e with C-3a (δ 116.08) and the phenolic carbon C-4 (148.21), respectively, allowed us to confirm the C(-1)−C(9a) and C(3)−C(3a) connectivities of the phenalene nucleus. Additionally,

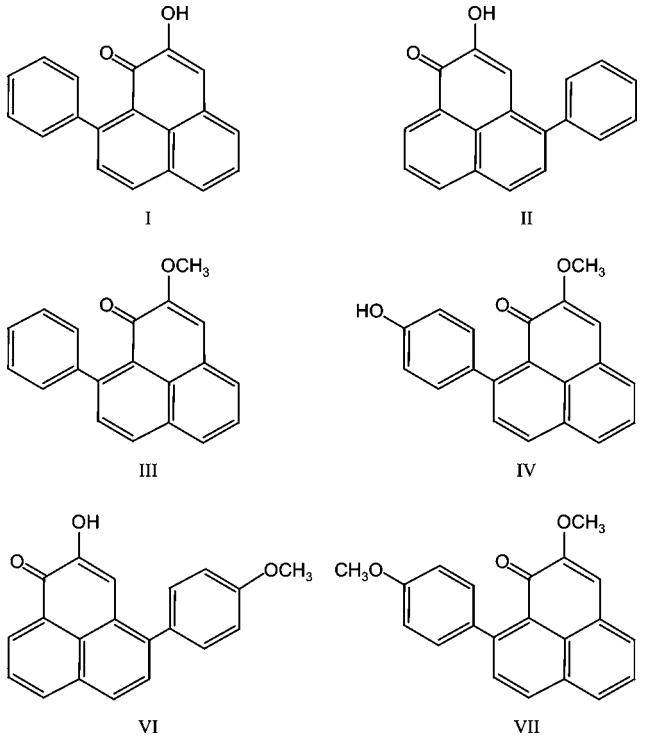

Figure 2. Structures of the known phenylphenalenone type compounds identified in the leaf fibers of abaca (*M. textilis*).

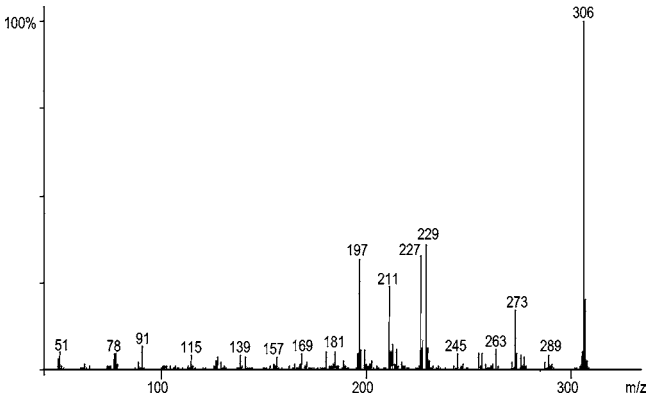

Figure 3. Mass spectra of peak V.

long-range correlations of H-2a, 2e (δ 2.12, δ 2.24), and H-1 with the *ipso* carbon C-1 (δ 144.54) located the monosubstituted phenyl ring at C-1. HSQC cross-peaks completed the assignments of the hydrogenated carbon atoms (**Table 1**).

The relative configuration of **V** was determined by the coupling constants of aliphatic part and from the observed NOESY cross peaks. The pseudo equatorial orientation of H-1 was established by the coupling constants (4.0, 2.5 Hz), and the large *trans*-axial coupling constant of H-2a with H-3a (13.0 Hz) indicated a pseudo-chair conformation of this ring. Consistently with these observations, H-2′, 6′ showed NOE interactions with the H-2e and H-3a indicating that these protons are oriented to the same side of the monosubstituted phenyl ring. Additional NOE cross-peaks were observed for H-7 with the methoxyl group and H-6. Therefore, the structure of the new natural compound was determined as (1*R*)-2,3-dihydro-4,9-dihydroxy-8-methoxy-1-phenylphenalene (**Figure 4**), and to the best of our knowledge, this is the first time that it has been described. Structurally, this compound is related to a phenylphenalenone type dimer isolated from *Anigozanthos flavidus*, a species of the Haemodoraceae plant family (*17*). Other dihydrophenalenes, structurally related to compound **V**, are also known in *M. acuminata* (*3*, *5*).

Table 1. ^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) Chemical Shifts for Compound **V** with *J* Values (Hz) in Parentheses

C/H no.	^{1}H	^{1}C
1	4.74 dd (4.0, 2.5)	37.84
2		28.95
2a	2.12 dddd (13.0, 13.0, 4.0, 4.0)	
2e	2.23 dddd (13.0, 4.0, 2.5, 2.5)	
3		17.99
3a	2.44 ddd (16.0, 13.0. 4.0)	116.08
3e	2.84 ddd (16.0, 4.0, 2.5)	
4-OH	4.65 bs	148.21
5	6.89 d (8.5)	114.91
6	7.44 d (8.5)	125.68
6a		123.71
7	6.98 s	104.57
8		145.57
9-OH	5.80 s	142.24
9a		117.64
9b		125.37
1′		144.53
2′, 6′	6.90 dd (7.0, 1.0)	127.88
4′	7.08 ddd (7.0, 7.0, 1.0)	125.92
3′, 5′	7.13 dd (7.0, 7.0)	128.11
$-OCH_3$	3.90 s	55.79

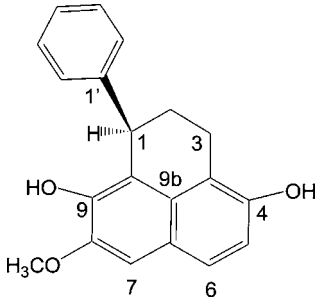

Figure 4. Structure of the novel compound, (1*R*)-2,3-dihydro-4,9-dihydroxy-8-methoxy-1-phenylphenalene (peak V), identified in the leaf fibers of abaca (*M. textilis*).

Phenylphenalenones represent a class of phenylpropanoid-derived natural products that occur in the Musaceae (genera *Musa* and *Ensete*) (*1*−*4*, *18*) as well as in other plant families such as the Haemodoraceae (*19*), Pontederiaceae (*20*), and Strelitziaceae (*21*). Phenylphenalenones from Musaceae and Haemodoraceae are of special interest because of their potential role as phytoalexins and phytoanticipins (*1*−*4*, *22*, *23*). However, despite their occurrence in different *Musa* species, including the banana plants (*M. acuminata* and *M. paradisiaca*), in which they have been demonstrated to be active as phytoalexins (*1*−*4*) and nematicides (*7*), this is the first time that phenylphenalenone compounds have been reported in abaca (*M. textilis*).

Banana plants (*Musa* sp., including *M. textilis*) are known to be affected by different pathogenic fungi such as *F. oxysporum* var. *cubensis* type 4 (casual agent of the Black Sigatoka disease) or *M. fijiensis* (responsible for the so-called Panama disease). Under colonization by these microorganisms, banana plants produce phenylphenalenone type phytoalexins (*1*−*4*). Because of their intense red or orange color, phenylphenalenones are visually detectable in pigmented roots and rhizomes, which seem to be the richest source of these natural products (*4*, *16*, *24*, *25*), although they have also been detected in the leaves of some *Musa* sp. infected with pathogenic fungi (*8*, *9*). Therefore, after the identification of phenylphenalenones in the abaca leaf fibers, it seems reasonable to assume that there is a strong correlation between the colored spots in abaca fibers and the presence of

these compounds in this parts of the fibers. Moreover, the presence of phenylphenalenones in the leaf fibers of abaca studied here is strong evidence that they have been attacked by these microorganisms and suffered from similar diseases as other *Musa* species. These diseases can drastically reduce the production of abaca fibers and also their quality. The presence of phenylphenalenone compounds may therefore be used as a parameter to assess the quality of the fiber.

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