

Phenylphenalenones from *Musa* cv. 'Thepanom' (BBB)

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Abstract: Chemical constituents of *Musa* cv. 'Thepanom' (BBB) or "praying hands" banana (Musaceae) were investigated by means of chromatographic techniques. A new natural product of the phenylphenalenone type, 3-hydroxy-4-phenyl-1*H*,3*H*-benzo[*de*]isochromen-1-one, and two known natural products, methoxyanigorufone and isoanigorufone, were isolated and identified by NMR spectroscopy and mass spectrometry. Additionally, by HPLC analysis, three condensed dimeric phenylphenalenones, namely anigorootin, 4'-hydroxyanigorootin, 4',4''-dihydroxyanigorootin, were identified.

Keywords: *Musa*; Musaceae; benzoisochromenones; phenylphenalenones.

1. Introduction

Plants of the genus *Musa* (family Musaceae), comprising bananas and plantains, are large perennial herbs growing from a sympodial rhizome [1]. They can be divided into different groups according to chromosome numbers and ploidy as well as characteristics contributed by two ancestors, *Musa acuminata* (genome type A) and *M. balbisiana* (genome type B). *Musa* spp. originated in Southeast Asia and nowadays are distributed throughout the tropics and sub-tropics [2]. In Thailand and India they are traditionally used to treat gastrointestinal disorders [3].



Figure 1. *Musa* cv. 'Thepanom' (BBB) or praying hands banana.

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Bananas and plantains have become economically important as food crops. Banana plantations face serious problems from devastating diseases, e.g. *Fusarium* Wilt and Black Sigatoka disease [4]. *Musa* cv. 'Thepanom' (BBB) or praying hands banana (originating from Philippines where it is known as Inabaniko) [5], due to the unique characteristics of its fruit (Fig. 1.) is used ornamentally, although the fruits are edible.

Phytochemical studies of the genus *Musa* reported the occurrence of phenylphenalenones and related structures [6-8]. Moreover, terpenoids [9] and flavonoids [10,11] have been described in *Musa* spp. There has been no previously reported phytochemical investigation of *Musa* cv. 'Thepanom' (BBB). As part of our phytochemical studies on *Musa* species, the chemical constituents of the *Musa* cultivar 'Thepanom' (genome type BBB) from Thailand are reported here.

2. Materials and Methods

2.1. Plant Material

A rhizome sample of *Musa* cv. "Thepanom" (BBB) (Musaceae) was collected in May 2004 from Pakchong Research Station, Nakhornratchasima, Thailand, where the plant is continuously cultivated. The specimen was identified by Mrs. Kulayanee Suwittawas.

2.2. Chromatographic and Spectroscopic Methods

Preparative HPLC: Merck-Hitachi LiChrograph HPLC system; column: LiChrospher RP18, 10 μ m, 250 \times 10 mm. Semipreparative HPLC: (Agilent series HP1100, binary pump G1312A; auto sampler G1313A; diode array detector G1315B, column: LiChrospher RP18, 5 μ m, 250 \times 4 mm. Mass spectra (ESIMS) were recorded on a Bruker Esquire 3000 ion trap mass spectrometer controlled via HyStar version 3.2.37. The accurate mass (HRESIMS) was recorded in positive ionization mode on a Micromass Quattro II tandem quadrupole mass spectrometer, Waters, Manchester, UK. ^1H NMR, HMQC, HMBC spectra were recorded on a Bruker AVANCE 500 NMR spectrometer equipped with a 5 mm TCI cryoprobe. UV-Vis spectra were recorded from an Agilent G1315B diode array detector during analytical HPLC in MeCN/H₂O (0.1% TFA) solvent mixture. The optical rotation was measured on a Jasco P-1030 polarimeter.

2.3. Analytical Data of 3-Hydroxy-4-phenyl-1H,3H-benzo[de]isochromen-1-one (**1**)

^1H NMR (500 MHz, acetone-*d*₆): δ 8.44 (1H, dd, $J_1 = 7.2$ Hz, $J_2 = 1.1$ Hz, H-9), 8.38 (1H, dd, $J_1 = 8.3$ Hz, $J_2 = 1.1$ Hz, H-7), 8.18 (1H, d, $J = 8.5$ Hz, H-6), 7.81 (1H, dd, $J_1 = 8.3$ Hz, $J_2 = 7.2$ Hz, H-8), 7.65 (2H, m, H-2'/6'), 7.64 (1H, d, $J = 8.5$ Hz, H-5), 7.55 (2H, m, H-3'/5'), 7.49 (1H, m, H-4'), 7.15 (1H, d, $J = 5.8$ Hz, 3-OH), 6.67 (1H, d, $J = 5.8$ Hz, H-3). ^{13}C NMR (125 MHz, acetone-*d*₆, chemical shifts extracted from HMQC and HMBC): δ 164.4 (C-9), 139.8 (C-4), 134.7 (C-7), 132.5 (C-1'), 132.4 (C-6a), 130.7 (C-9), 130.4 (C-5), 130.4 (C2'/6'), 129.5 (C-6), 129.4 (C-3'/5'), 129.0 (C-4'), 128.5 (C-9b), 127.3 (C-8), 127.2 (C-3a), 121.7 (C-9a), 95.2 (C-3). UV (MeCN/H₂O): λ_{max} 225, 250, 321 nm. HRESIMS: m/z 299.0677 (calc. for C₁₈H₁₂O₃Na 299.0684) Optical rotation: $[\alpha]_{\text{D}}^{22} = +17.2^\circ$ (c 0.00022, MeOH).

3. Results and Discussion

Fresh rhizome material (100 g) of *Musa* cv. "Thepanom" (BBB) was exhaustively extracted with 96% EtOH. The combined extracts were evaporated to a final volume of 50 ml and then partitioned with CH₂Cl₂ (50 ml \times 2). The CH₂Cl₂ fraction was evaporated to dryness (70 mg) by rotary evaporator ($\leq 50^\circ\text{C}$) and further separated on a manually packed RP-18 column (\varnothing 2 \times 4 cm), eluted with MeOH-H₂O mixtures (50%, 70% and pure MeOH). The fraction eluted with 70% MeOH in H₂O

(11 mg) was further separated by preparative HPLC. A linear gradient of MeCN in H₂O was used as a mobile phase (0 min: 20% MeCN, 60 min: 90% MeCN, 65 min: 100% MeCN, 70 min: 20% MeCN) at a flow rate of 3.5 mL min⁻¹ and UV detection at 254 nm. The fraction collected at 41-48 min was further purified by semipreparative HPLC using a 4 mm column). A linear gradient of MeCN in H₂O containing 0.1% trifluoroacetic acid (TFA) was used as a mobile phase (0 min: 40% MeCN, 40 min: 50% MeCN, 45 min: 100% MeCN, 50 min: 40% MeCN) at a flow rate of 1.2 mL min⁻¹. Compounds **1** (0.3 mg), **2** (0.1 mg) and **3** (0.1 mg) were eluted at *R*_t 14.4, 27.1 and 31.0 min, respectively. Structure elucidation of the isolated compounds **1-3** was accomplished by spectroscopic techniques.

NMR spectra of compounds **2** and **3** and UV/Vis absorptions corresponded to reported data of 2-methoxy-9-phenyl-1*H*-phenalen-1-one (methoxyanigorufone) [6] and 2-hydroxy-4-phenyl-1*H*-phenalen-1-one (isoanigorufone, 4'-dehydroxyirenonone) [12]. Furthermore, NMR spectra in acetone-*d*₆ matched data of authentic references in our NMR data base. ESIMS of compound **2** (*m/z* 273 [M+H]⁺) and **3** (*m/z* 287 [M+H]⁺) further confirmed the two structures.

The fraction eluted with pure MeOH (37 mg) was analysed by HPLC using a linear gradient of MeCN in H₂O containing 0.1% TFA (0 min: 30% MeCN, 60 min: 70% MeCN, 65 min: 100% MeCN, 70 min: 30% MeCN) at a flow rate of 1 mL min⁻¹. Compounds **4-6** eluted at *R*_t 53.8, 43.8 and 33.0 min, respectively. Comparisons between the retention times and UV absorbance spectra with those of authentic compounds previously reported for *Anigozanthos flavidus* and *Musa acuminata* [13] allowed compounds **4-6** to be identified as the condensed dimeric phenylphenalenones, anigorootin (**4**), 4'-hydroxyanigorootin (**5**) and 4',4''-dihydroxyanigorootin (**6**).

Compound **1**, a new natural product, was obtained as a white powder. Its chemical structure was identified using 1D and 2D NMR (¹H, ¹H-COSY, HSQC, HMBC) and MS. The HRESIMS showed a [M+Na]⁺ ion at *m/z* 299.0677 (calc. for C₁₈H₁₂O₃Na 299.0684) corresponding to the molecular formula of C₁₈H₁₂O₃. The ¹H NMR (acetone-*d*₆) exhibited signals of eleven non-exchangeable protons. The ABX spin system at δ 7.64 (H-5) and 8.18 (H-6) and an AMX spin system, appearing at δ 8.38 (H-7), 7.81 (H-8) and 8.44 (H-9), are characteristic of phenylphenalenones, which do not carry an additional substituent in rings B and C. The multiplet signals at δ 7.65 (2H, H-2'/6'), 7.55 (2H, H-3'/5') and 7.49 (1H, H-4') were assigned to the monosubstituted phenyl ring. The ¹H NMR spectrum in acetone-*d*₆ displayed two doublets at δ 6.67 (H-3) and 7.15 (3-OH) that were coupled with each other as shown by their coupling constant *J* = 5.8 Hz. The signal at δ 7.15 disappeared and the doublet at δ 6.67 collapsed to a singlet signal when the ¹H NMR of compound **1** was measured in MeOH-*d*₄. This indicated that the doublet signal at δ 7.15 was from an exchangeable proton assignable to 3-OH.

Correlations in the HMBC spectrum of H-7 with C-6 (δ 129.5), C-9 (δ 130.7) and C-9b (δ 128.5), H-6 with C-4 (δ 139.8), C-7 (δ 134.7) and C-9b, H-9 with C-9b and C-7, H-5 with C-3a (δ 127.2) and C-6a (δ 132.4), H-8 with C-6a and C-9a (δ 121.7) confirmed the connection of the ABX and AMX spin systems and thereby the naphthalene moiety (rings B and C). The long-range correlation signals of H-3 with the carbonyl carbon C-1 (δ 164.4) and C-9b, 3-OH with C-3, and H-9 with C-1 established the structure of the 3-hydroxy-benzo[*de*]isochromen-1-one moiety. The HMBC cross signals of H-2'/6' with C-4 (δ 139.8) and H-5 with C-1' (δ 132.5) indicated the phenyl ring at C-4. From these data, compound **1** was identified as 3-hydroxy-4-phenyl-1*H*,3*H*-benzo[*de*]isochromen-1-one. A similar natural product, namely 3-hydroxy-4-(4'-hydroxyphenyl)-1*H*,3*H*-benzo[*de*]isochromen-1-one (**7**), was reported as a racemic mixture [7].

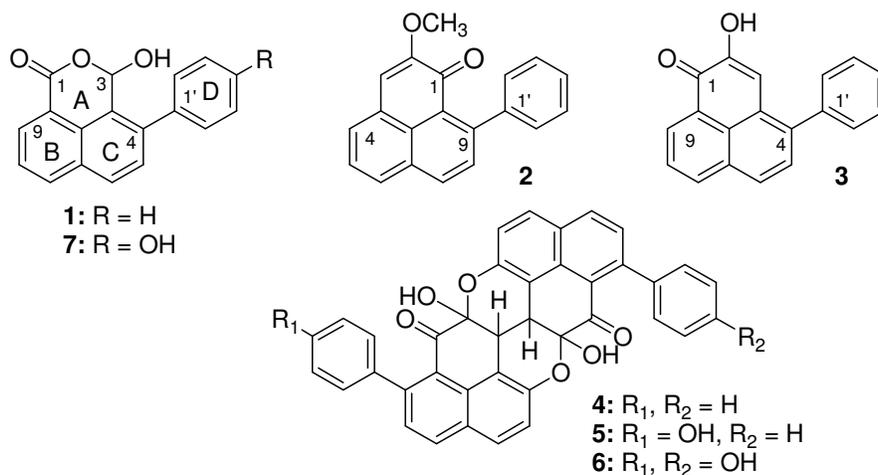


Figure 2. Natural products from *Musa* cv. "Thepanom", (BBB). **1:** 3-Hydroxy-4-phenyl-1*H*,3*H*-benzo[*de*]isochromen-1-one, **2:** Methoxyanigorufone, **3:** Isoanigorufone, **4:** Anigorootin, **5:** 4'-Hydroxyanigorootin, **6:** 4',4''-Dihydroxyanigorootin. The numbering convention for phenylphenalenones was also used for compound **1**. Compound **7** has been isolated from unripe fruits of *M. acuminata* in an earlier study [7].

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