

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/11079680>

Constituents of *Musa x paradisiaca* cultivar with the potential to induce the phase II enzyme, quinone reductase.

ARTICLE in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · NOVEMBER 2002

Impact Factor: 2.91 · Source: PubMed

CITATIONS

8

READS

56

12 AUTHORS, INCLUDING:



Park Eun Jung

Yonsei University

136 PUBLICATIONS 3,323 CITATIONS

SEE PROFILE



Michael Hawthorne

IIT Research Institute (IITRI)

33 PUBLICATIONS 1,092 CITATIONS

SEE PROFILE



Bernard Santarsiero

University of Illinois at Chicago

192 PUBLICATIONS 5,496 CITATIONS

SEE PROFILE



Rajendra Mehta

Illinois Institute of Technology

223 PUBLICATIONS 10,431 CITATIONS

SEE PROFILE

Constituents of *Musa* × *paradisiaca* Cultivar with the Potential
To Induce the Phase II Enzyme, Quinone Reductase

DAE SIK JANG,[†] EUN JUNG PARK,[†] MICHAEL E. HAWTHORNE,[‡]
JOSE SCHUNKE VIGO,[§] JAMES G. GRAHAM,[†] FERNANDO CABIESES,[§]
BERNARD D. SANTARSIERO,[†] ANDREW D. MESECAR,[†] HARRY H. S. FONG,[†]
RAJENDRA G. MEHTA,[‡] JOHN M. PEZZUTO,[†] AND A. DOUGLAS KINGHORN^{*,†}

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal
Chemistry and Pharmacognosy, and Center for Pharmaceutical Biotechnology, College of Pharmacy,
and Department of Surgical Oncology, College of Medicine, University of Illinois at Chicago,
Chicago, Illinois 60612, and Instituto Nacional de Medicina Tradicional (INMETRA),
Ministerio de Salud, Jesus Maria, Lima, Peru

A new bicyclic diarylheptanoid, *rel*-(3*S*,4*aR*,10*bR*)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4*a*,5,6,10*b*-tetrahydro-3*H*-naphtho[2,1-*b*]pyran (**1**), as well as four known compounds, 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene (**2**), hydroxyanigorufone (**3**), 2-(4-hydroxyphenyl)naphthalic anhydride (**4**), and 1,7-bis(4-hydroxyphenyl)hepta-4(*E*),6(*E*)-dien-3-one (**5**), were isolated from an ethyl acetate-soluble fraction of the methanol extract of the fruits of *Musa* × *paradisiaca* cultivar, using a bioassay based on the induction of quinone reductase (QR) in cultured Hepa1c1c7 mouse hepatoma cells to monitor chromatographic fractionation. The structure and relative stereochemistry of compound **1** were elucidated unambiguously by one- and two-dimensional NMR experiments (¹H NMR, ¹³C NMR, DEPT, COSY, HMQC, HMBC, and NOESY) and single-crystal X-ray diffraction analysis. Isolates **1**–**5** were evaluated for their potential cancer chemopreventive properties utilizing an in vitro assay to determine quinone reductase induction and a mouse mammary organ culture assay.

KEYWORDS: *Musa* × *paradisiaca* cultivar; Musaceae; diarylheptanoids; phenylphenalenones; X-ray diffraction analysis; quinone reductase; mouse mammary organ culture assay; cancer chemoprevention

INTRODUCTION

Musa × *paradisiaca* L. (Musaceae), with orange-yellow flowers and long, narrow, starchy, edible fruits, is the common name for the cultivar French Plantain, which is of hybrid origin (*Musa acuminata* × *Musa balbisiana* Colla) (1). Previous phytochemical investigations on plants of the genus *Musa* have resulted in the isolation of phenylphenalenones (2), triterpenes (3, 4), flavonoids (5, 6), and sterols (7, 8). However, there is only a single report on the phytochemistry of *Musa* × *paradisiaca*, in which some anthocyanins were described (9). In our ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents from plants (10, 11), the fruits of *Musa* × *paradisiaca* cultivar were chosen for a more detailed investigation, since the ethyl acetate-soluble fraction of the methanol extract significantly induced the enzyme quinone reductase (QR) in cultured Hepa1c1c7 (mouse hepatoma) cells. Phase II drug-metabolizing enzymes,

such as QR and glutathione *S*-transferase in rodent tissues, are primarily responsible for the metabolic detoxification of chemical carcinogens and other harmful oxidants. Therefore, induction of QR is a major mechanism of protection against tumor initiation (10, 12).

Bioassay-guided fractionation of the ethyl acetate-soluble fraction of the methanol extract of *Musa* × *paradisiaca* cultivar using the QR induction assay led to the isolation and characterization of a new bicyclic diarylheptanoid, *rel*-(3*S*,4*aR*,10*bR*)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4*a*,5,6,10*b*-tetrahydro-3*H*-naphtho[2,1-*b*]pyran (**1**), and the identification of four constituents of previously known structure, 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene (**2**), hydroxyanigorufone (**3**), 2-(4-hydroxyphenyl)naphthalic anhydride (**4**), and 1,7-bis(4-hydroxyphenyl)-hepta-4(*E*),6(*E*)-dien-3-one (**5**). These isolates were evaluated for their potential cancer chemopreventive properties in the Hepa1c1c7 model. Selected compounds (**1** and **3**) were then chosen for evaluation in a mouse mammary organ culture assay. The structure elucidation of **1** and the biological evaluation of **1**–**5** are described herein.

* To whom correspondence should be addressed. Tel.: +01-312-996-0914. Fax: +01-312-996-7107. E-mail: kinghorn@uic.edu.

[†] College of Pharmacy, University of Illinois at Chicago.

[‡] College of Medicine, University of Illinois at Chicago.

[§] Instituto Nacional de Medicina Tradicional (INMETRA).

MATERIALS AND METHODS

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were recorded on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as the internal standard. MS and HRMS were carried out on a Finnigan MAT 90/95 sector-field mass spectrometer. Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F₂₅₄ (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H₂SO₄ reagent (Aldrich, Milwaukee, WI) followed by charring at 110 °C for 5–10 min. Silica gel (Merck 60A, 70–230 or 200–400 mesh ASTM) and Sorbisil C₁₈ reversed-phase silica gel (Sigma, St. Louis, MO) were used for column chromatography. Preparative TLC was performed on Kieselgel 60 F₂₅₄ (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

X-ray crystallographic analysis data collection for compound **1** was carried out on an Enraf-Nonius Kappa CCD area detector equipped with a rotating anode X-ray generator and Mo K α radiation (13). The direct methods program SIR-92 (14) was used to locate the non-hydrogen atoms. Repeated cycling with least-squares refinement on F² with SHELX-97 (15) and difference Fourier maps yielded the final structure, and were useful in identifying hydrogen atom positions. All non-hydrogen atoms were refined with anisotropic Gaussian displacement parameters. The ORTEP (16) diagram was drawn with 50% probability ellipsoids.

Plant Material. The fruits of *Musa × paradisiaca* L. cultivar (syn. *Musa × sapientum* L.; *Musa acuminata* × *Musa balbisiana* Colla) (known locally as “huiracucha platano”) were collected from a plantation in Loreto Province, Peru, in October 1998 by two of us (J.S.V. and J.G.G.). A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. Schunke 14529).

Biological Assays for the Induction of Quinone Reductase with Cultured Mouse Hepatoma Cells. For the evaluation of plant extracts, fractions, and pure isolates as inducers of quinone reductase (QR), cultured mouse Hepalclc7 cells (supplied by J. P. Whitlock, Jr., Stanford University, Stanford, CA) were used as described previously (17–19). In brief, 2×10^3 cells/mL were added to the wells of 96-well plates (200 μ L/well) and incubated for 24 h. Following replacement of the medium, test compounds, dissolved in 10% DMSO, were added (eight serial 2-fold dilutions in a final concentration range of 0.15–20 μ g/mL), and the plates were incubated for an additional 48 h. QR induction activity was determined by assessing the NADPH-dependent menadiol-mediated reduction of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] to a blue formazan. The amount of protein was determined by staining with crystal violet in an identical set of test plates. The extent of induction of QR activity was calculated from the ratio of specific enzyme activity of compound-treated cells in comparison with a solvent control, and CD values (micrograms per milliliter), representing the concentration to double QR induction, were generated. Compounds with CD values of <10 μ g/mL were considered active. IC₅₀ values (micrograms per milliliter) (half-maximal inhibitory concentration of cell viability) were divided by CD values to obtain a chemopreventive index (CI) value (18).

Mouse Mammary Organ Culture Assay. This assay was carried out according to an established protocol (20). In brief, BALB/c female mice (4 weeks old; Charles River, Wilmington, MA) were pretreated for 9 days with 1 μ g of estradiol and 1 mg of progesterone. On the 10th day, the mice were sacrificed and the second pair of thoracic mammary glands was dissected on silk and transferred to 60 mm culture dishes containing 5 mL of Waymouth's 752/1 MB medium supplemented with streptomycin, penicillin, and L-glutamine. The glands were incubated for 10 days (37 °C, 95% O₂ and 5% CO₂) in the presence of growth-promoting hormones (5 μ g of insulin, 5 μ g of prolactin, 1 μ g of aldosterone, and 1 μ g of hydrocortisone per milliliter of medium). Glands were exposed to 2 μ g/mL 7,12-dimethylbenz[*a*]anthracene

(DMBA) between 72 and 96 h. After their exposure, glands were rinsed and transferred to new dishes with fresh medium. We then permitted the fully differentiated glands to regress by withdrawing all hormones except insulin for 14 additional days. Test compounds were present in the medium during days 1–10 of culture; mammary glands were scored for incidence of lesions.

Extraction and Isolation. The dried and milled plant material (1.64 kg) was extracted with 4 L of MeOH three times by maceration. The extracts were combined and concentrated in vacuo at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with 1 L of petroleum ether three times to afford a petroleum ether-soluble syrup (D001) on drying. Next, the aqueous methanol extract was concentrated and suspended in H₂O (2 L) and partitioned again with 1 L of EtOAc three times to give an EtOAc-soluble extract (D002) and an aqueous residue (D003). The CD values (micrograms per milliliter) of the MeOH extract and the solvent partitions (D001–D003) were 1.7, >10, 1.4, and >10, respectively.

On the basis of the activity results described above, the EtOAc-soluble extract (D002, 3.4 g) was chromatographed over silica gel as a stationary phase using a CHCl₃/MeOH gradient (from 1:0 to 1:1, v/v) as mobile phase to afford 12 pooled fractions (F004–F015). Of these, fractions F008 and F010 showed the most potent QR-inducing activity (CD values of 2.7 and 3.5 μ g/mL, respectively). Fraction F008 [eluted with CHCl₃/MeOH (49:1, v/v), 195 mg] was further fractionated by reversed-phase low-pressure liquid chromatography over C₁₈ silica gel (gradient from 70% MeOH in H₂O to 100% MeOH as the solvent), resulting in nine subfractions (F016–F024). The new compound **1** (26 mg, 0.0016%, in petroleum ether/EtOAc) and the phenylphenalenone **3** (14 mg, 0.00085%, in MeOH) were obtained from fractions F023 and F020, respectively, by recrystallization. Additional chromatographic separation of fraction F021 was carried out by preparative TLC (15% acetone/CHCl₃ as the developing solvent; *R*_f = 0.64) to afford phenylphenalenone **4** (1.2 mg, 0.000073%).

Another active fraction, F010 [eluted with CHCl₃/MeOH (97:3, v/v), 185 mg], was chromatographed over silica gel with CHCl₃/acetone (99:1 → 4:1, v/v) to produce subfractions F028–F033. The diarylheptanoid **5** (2.1 mg, 0.00013%) was isolated from fraction F029 by preparative TLC (10% EtOH/CHCl₃ as the developing solvent; *R*_f = 0.52). Another phenylphenalenone **2** (0.8 mg, 0.000049%) was isolated from fraction F030 by preparative TLC (40% EtOAc/*n*-hexane as the developing solvent; *R*_f = 0.56).

rel-(3S,4aR,10bR)-8-Hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4a,5,6,10b-tetrahydro-3H-naphtho[2,1-*b*]pyran (1) was obtained as colorless crystals: mp 195–197 °C; [α]_D²⁰ –292.8° (*c* 0.77, MeOH); UV (EtOH) λ_{max} (log ϵ) 240 (3.28), 269 (3.23), 294 nm (3.40); IR ν_{max} (NaCl) 3365, 3021, 2926, 2948, 1607, 1510, 1446, 1363, 1265, 1236, 1110, 1056, 832, 756 cm^{–1}; EIMS *m/z* (relative intensity) 324 ([M]⁺, 51), 173 (100), 137 (49), 121 (11); HRCIMS *m/z* 325.1466 ([M + H]⁺, C₂₀H₂₁O₄, calcd 325.1440). For ¹H NMR, ¹³C NMR, and HMBC data, see Table 1.

X-ray Crystallography of Compound 1. A colorless crystal was obtained from petroleum ether/EtOAc (1:1) and selected for data collection. The crystal size was 0.17 mm × 0.30 mm × 0.35 mm. Cell parameters were as follows: *a* = 7.8164(2) Å, *b* = 8.4462(2) Å, *c* = 13.1755(4) Å, β = 91.8028(12)°, *V* = 869.40(4) Å³, *Z* = 2, *D*_{calc} = 1.308 g/cm³, *T* = 150(2) K, λ = 0.71073 Å, *P*₂₁ space group, μ (Mo K α) = 0.093 mm^{–1}, and *F*(000) = 364.0; refinement with 10 277 reflections (3562 with *I* > 2 σ) led to final *R*, *R* (all), and GOF values of 0.0331, 0.0388, and 1.057, respectively. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 184410. Copies of the data can be obtained, free of charge, upon application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.

1,2-Dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene (2) was obtained as a pale yellow solid: [α]_D²⁰ –20.0° (*c* 0.04, CHCl₃); EIMS *m/z* (relative intensity) 322 ([M]⁺, 54), 304 (21), 287 (100), 271 (20), 202 (20), 180 (12). ¹H and ¹³C NMR data were in agreement with the reported literature values (21, 22).

Hydroxyanigorufone (3) was obtained as orange crystals: mp 201–204 °C; EIMS *m/z* (relative intensity) 287 ([M – H]⁺, 100), 271 (19),

Table 1. NMR Data for Compound 1

position	δ_c^a	δ_H multiplicity [J (Hz)] ^b	HMBC correlations ^b
1	130.4, d	6.21, ddd (10.3, 3.8, 2.1)	C-3, C-4a, C-10a, C-10b
2	128.6, d	5.82, dt (10.3, 2.1)	C-3, C-10b
3	73.7, d	5.07, d (2.1)	C-4a, C-1', C-2'/6'
4a	69.8, d	4.14, m	C-3, C-5, C-6, C-10a
5	27.3, t	1.80, m; 1.99, m	C-4a, C-6, C-6a, C-10b
6	27.0, t	2.52, m; 2.79, m	C-4a, C-5, C-6a, C-7, C-10a
6a	130.0, s		
7	116.0, d	6.53, s	C-6, C-6a, C-8, C-9, C-10a
8	145.8, s		
9	147.9, s		
10	112.9, d	6.82, s	C-8, C-9, C-10b
10a	129.9, s		
10b	38.3, d	3.40, brs	C-10a
1'	133.2, s		
2'/6'	130.6, d	7.23, d (8.5)	C-3, C-1', C-4', C-3'/C-5'
3'/5'	116.2, d	6.78, d (8.5)	C-1', C-2'/C-6', C-4'
4'	158.4, s		
OMe-9	56.7, q	3.83, s	C-19

^a The spectrum was recorded in CD₃OD at 75 MHz, and values are reported in parts per million relative to TMS. Multiplicities were determined by a DEPT ¹³C NMR experiment, and are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet. ^b Spectra were recorded in CD₃OD at 300 MHz. Coupling constants are reported in hertz. Multiplicities are as follows: ddd, doublet of double doublets; dt, doublet of triplets; m, multiplet; brs, broad singlet. Assignments are based on COSY, HMQC, and HMBC experiments.

201 (11). ¹H and ¹³C NMR data were in agreement with the reported literature values (23).

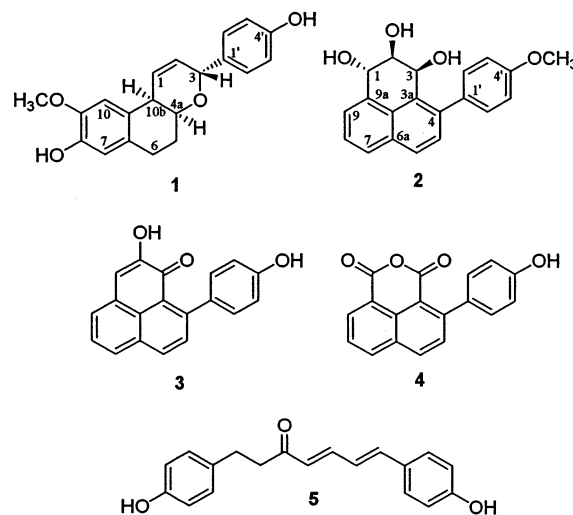
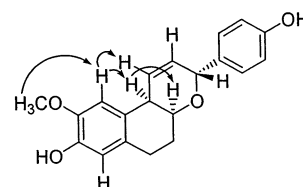
2-(4-Hydroxyphenyl)naphthalic anhydride (4) was obtained as a yellowish powder: mp 249–252 °C; EIMS *m/z* (relative intensity) 290 ([M]⁺, 100), 246 (17), 218 (36), 189 (43). ¹H and ¹³C NMR data were in agreement with the reported literature values (24).

1,7-Bis(4-hydroxyphenyl)hepta-4(E),6(E)-dien-3-one (5) was obtained as a yellow amorphous solid: EIMS *m/z* (relative intensity) 294 ([M]⁺, 58), 200 (22), 187 (54), 173 (100), 146 (66), 107 (64). ¹H and ¹³C NMR data were in agreement with the reported literature values (25).

RESULTS AND DISCUSSION

Purification of the ethyl acetate-soluble fraction of the methanol extract of the fruits of *Musa × paradisiaca* cultivar, using a quinone reductase assay to monitor fractionation, led to the isolation of a new diarylheptanoid (**1**), along with four known compounds, 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene (**2**) (21, 22), hydroxynigorufone (**3**) (23), 2-(4-hydroxyphenyl)naphthalic anhydride (**4**) (24), and 1,7-bis(4-hydroxyphenyl)-hepta-4(E),6(E)-dien-3-one (**5**) (Figure 1) (25). Compound **5** has not been isolated from a *Musa* species previously. The structures of the known compounds were identified by physical (melting point and [α]_D) and spectroscopic (¹H NMR, ¹³C NMR, two-dimensional NMR, and MS) measurement and by comparison with published values.

Compound **1** was obtained as colorless crystals and gave a protonated molecular ion ([M + H]⁺) by HRCIMS, consistent with an elemental formula of C₂₀H₂₁O₄. Assignments of the resonances of all of the hydrogen and carbon atoms in the molecule (Table 1) were made by application of one- and two-dimensional homo- and heteronuclear NMR experiments (¹H NMR, ¹³C NMR, DEPT, COSY, NOESY, HMQC, and HMBC). Thus, ¹³C NMR and DEPT experiments with **1** showed the presence of signals for 11 methine groups, with two of them (δ 73.7 and 69.8) bearing oxygen atoms, as well as two methylene groups (δ 27.3 and 27.0), one methoxyl group (δ 56.7), and six quaternary carbons, with three of the latter (δ 145.8, 147.9, and 158.4) also bearing an oxygen atom. The ¹H NMR spectrum

Figure 1. Structures of compounds 1–5 isolated from *Musa × paradisiaca*.Figure 2. Selected correlations observed in the NOESY spectrum of **1**.

showed two ortho-coupled doublets centered at δ 6.78 (2H, J = 8.5 Hz) and δ 7.23 (2H, J = 8.5 Hz), indicative of the protons of a para-substituted benzene ring. Two additional singlet aromatic proton signals at δ 6.53 and 6.82 were also observed, suggesting the presence of an additional tetrasubstituted benzene ring. Signals for an isolated double bond were observed as a double double-doublet resonance at δ 6.21 (1H, J = 10.3, 3.8, 2.1 Hz) and a double-triplet resonance at δ 5.82 (1H, J = 10.3, 2.1 Hz), and demonstrated that this double bond was connected to methine carbons C-3 (δ_H 5.07, d, J = 2.1 Hz, H-3) and C-10b (δ_H 3.40, brs, H-10b). An A₂M₂X spin system resonance, with the A₂ part represented by the multiplet signals at δ 2.52 and δ 2.79 (each 1H, H-6), the M₂ part by the multiplet signals at δ 1.80 and 1.99 (each 1H, H-5), and the X part by one-proton multiplet signals at δ 4.14 (H-4a), was also observed in the high-field region of the ¹H NMR spectrum.

From the HMBC spectrum, it was inferred that compound **1** contains a tetrahydronaphthopyran moiety, in which the double bond is connected to a para-substituted benzene ring bearing a hydroxyl group and the A₂M₂X spin system is connected to the tetrasubstituted benzene ring having a hydroxyl group and a methoxyl group. The positions of the hydroxyl group and methoxyl group in the tetrasubstituted benzene ring were confirmed, and the relative configurations of the protons (H-10b, H-4a, and H-3) were determined on the basis of the key NOESY correlations from δ_H 3.83 (3H, s, MeO-9) to δ_H 6.82 (1H, s, H-10), and from δ_H 3.40 (H-10b) to δ_H 4.14 (1H, m, H-4a) (Figure 2). All of these data were in accordance with the assignment of **1** as *rel*-(3*S*,4*aR*,10*bR*)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4*a*,5,6,10*b*-tetrahydro-3*H*-naphtho[2,1-*b*]pyran, and its structure and stereochemistry were confirmed unambiguously by X-ray diffraction analysis (Figure 3). Although the numbering system of **1** used in Figure 3 does not follow that of *Chemical Abstracts*, this molecule is arranged in the same orientation as shown in Figures 1 and 2. To the best of our knowledge, there is just one prior report of a diarylheptanoid

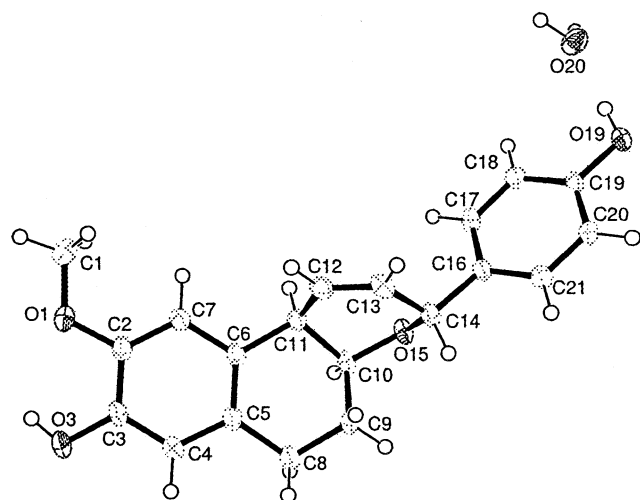


Figure 3. X-ray ORTEP structure of compound 1.

Table 2. Biological Activity of Compounds 1–5 from *Musa × paradisiaca* in the Quinone Reductase (QR) Induction and Mouse Mammary Organ Culture (MMOC) Bioassays

compound	QR ^a			MMOC (%) ^b
	CD (μg/mL)	IC ₅₀ (μg/mL)	CI	
1	9.3	>20	>2.2	55.6 (36.5)
2	5.3	>20	>3.8	ND ^c
3	3.8	6.5	1.7	63.0 (44.4)
4	>10	4.0	<0.4	ND
5	1.9	12.2	6.4	ND
sulforaphane ^d	0.087	2.1	24.1	83.7

^a QR activity was determined with the designated cell lines as described in Materials and Methods. CD is the concentration required to double QR activity. IC₅₀ is the concentration inhibiting cell growth by 50%. CI is the chemoprevention index (=IC₅₀/CD). ^b Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Selected compounds from *M. sapientum* were tested at concentrations of 10 and 1 μg/mL (in parentheses). On the basis of historical controls, inhibition of >60% (at 10 μg/mL) is considered significant. ^c Not determined since the amount of available compound was insufficient. ^d Sulforaphane was used as a positive control, and was tested at a concentration of 1 μg/mL (18, 19).

functionalized in a similar manner, which was isolated from *Alnus hirsuta* Turcz. (Betulaceae) (26).

The potential of compounds 1–5 to induce QR activity in Hepalcl7 cells is summarized in Table 2. The known compound 2-(4-hydroxyphenyl)naphthalic anhydride (4) was cytotoxic in this test system. However, all of the other four compounds significantly induced QR activity, with CD values ranging from 1.9 to 9.3 μg/mL. Of these, 1,7-bis(4-hydroxyphenyl)hepta-4(*E*),6(*E*)-dien-3-one (5) exhibited the most potent QR-inducing activity (CD value of 1.9 μg/mL), although the amount that was obtained was insufficient for further biological testing. Among the three phenylphenalones (2–4), hydroxy-anigorufone (3) was the most active (CD value of 3.8 μg/mL), whereas 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene (2) was the least cytotoxic (IC₅₀ > 20 μg/mL and CI value > 3.8). Compounds 1 and 3 were chosen for evaluation in a mouse mammary organ culture assay to evaluate the potential of inhibiting carcinogen-induced preneoplastic lesion formation (20). As a result, 2-(4-hydroxyphenyl)naphthalic anhydride (3) was shown to mediate a significant response (63% inhibition at 10 μg/mL), and the new compound 1 was marginally active (55.6% inhibition at 10 μg/mL) (Table 2). Thus, compound 3 will be considered further for its potential as a

cancer chemopreventive agent through additional biological evaluation.

ACKNOWLEDGMENT

We thank Dr. Djaja D. Soejarto, College of Pharmacy, University of Illinois at Chicago (UIC), for taxonomic assistance, Dr. J. A. (Art) Anderson, Research Resources Center, UIC, and Dr. K. Fagerquist, Mass Spectrometry Facility, Department of Chemistry, University of Minnesota (Minneapolis, MN), for the mass spectrometric data, and Dr. Philip E. Fanwick, Department of Chemistry, Purdue University (West Lafayette, IN), for the X-ray diffraction data. We are grateful to Dr. R. A. Kleps of the Research Resources Center, UIC, for facilitating the running of the 500 MHz NMR instrument.

Supporting Information Available: Experimental details of the X-ray analysis of compound 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- Argent, G. C. G. Musaceae. In *The European Garden Flora. Volume II, Monocotyledons (Part II) Juncaceae to Orchidaceae*; Walters, S. M., Brady, A., Brickell, C. D., Cullen, J., Green, P. S., Lewis, J., Matthews, V. A., Webb, D. A., Yeo, P. F., Alexander, J. C. M., Eds.; Cambridge University Press: Cambridge, U.K., 1984; pp 117–119.
- Luis, J. G.; Echeverri, F.; Quinones, W.; Brito, I.; Lopez, M.; Torres, F.; Cardona, G.; Aguiar, Z.; Pelaez, C.; Rojas, M. Irenolone and emenolone: Two new types of phytoalexin from *Musa paradisiaca*. *J. Org. Chem.* **1993**, *58*, 4306–4308.
- Dutta, P. K.; Das, A. K.; Banerji, N. A tetracyclic triterpenoid from *Musa paradisiaca*. *Phytochemistry* **1983**, *22*, 2563–2564.
- Banerji, N.; Sen, A. K.; Das, A. K. A new 9,19-cyclotriterpene from flowers of *Musa paradisiaca* (banana). *Indian J. Chem., Sect. B* **1982**, *21*, 387–388.
- Lewis, D. A.; Fields, W. N.; Shaw, G. P. A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum* L. var. *paradisiaca*) protects the gastric mucosa from aspirin-induced erosions. *J. Ethnopharmacol.* **1999**, *65*, 283–288.
- Ali, M.; Bhutani, K. K. Flavan-3,4-diols from *Musa sapientum* seeds. *Pharmazie* **1993**, *48*, 455–456.
- Akihisa, T.; Shimizu, N.; Tamura, T.; Matsumoto, T. (24*S*)-14 α -(24-Dimethyl-9 β ,19-cyclo-5 α -cholest-25-en-3 β -ol): A new sterol and other sterols in *Musa sapientum*. *Lipids* **1986**, *21*, 494–497.
- Ghosal, S. Steryl glycosides and acyl steryl glycosides from *Musa paradisiaca*. *Phytochemistry* **1985**, *24*, 1807–1810.
- Pazmiño-Durán, E. A.; Giusti, M. M.; Wrolstad, R. E.; Glória, B. A. Anthocyanins from banana bracts (*Musa × paradisiaca*) as potential food colorants. *Food Chem.* **2001**, *73*, 327–332.
- Pezzuto, J. M. In *Recent Advances in Phytochemistry, Vol. 29, Phytochemistry of Medicinal Plants*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; pp 19–44.
- Kinghorn, A. D.; Fong, H. H. S.; Farnsworth, N. R.; Mehta, R. G.; Moon, R. C.; Moriarty, R. M.; Pezzuto, J. M. Cancer chemopreventive agents discovered by activity-guided fractionation: A review. *Curr. Org. Chem.* **1998**, *2*, 597–612.
- Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403.
- Instrument located at the X-ray Laboratory, Chemistry Department, 234 Wetherall Hall, Purdue University, West Lafayette, IN 47907-1393, and operated by Dr. Philip E. Fanwick.
- Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. SIR92, A Program for Crystal Structure Solution. *J. Appl. Crystallogr.* **1993**, *26*, 343–350.

- (15) Sheldrick, G. *SHELXTL*, version 5.1; Bruker Analytical X-ray Systems: Madison, WI.
- (16) Farrugia, L. J. ORTEP3 for Windows. *J. Appl. Crystallogr.* **1997**, *30*, 565.
- (17) Prochaska, H. J.; Santamaria, A. B. Direct measurement of NAD(PH):quinone reductase from cells in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **1988**, *169*, 328–336.
- (18) Gerhäuser, C.; You, M.; Liu, J.; Moriarty, R. M.; Hawthorne, M.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res.* **1997**, *57*, 272–278.
- (19) Chang, L. C.; Gerhäuser, C.; Song, L.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. Activity-guided isolation of constituents of *Tephrosia purpurea* with the potential to induce the phase II enzyme, quinone reductase. *J. Nat. Prod.* **1997**, *60*, 869–873.
- (20) Mehta, R. G.; Moon, R. C. Characterization of effective chemopreventive agents in mammary gland *in vitro* using an initiation-promotion protocol. *Anticancer Res.* **1991**, *11*, 593–596.
- (21) Luis, J. G.; Fletcher, W. Q.; Echeverri, F.; Grillo, T.; Perales, A.; González, J. A. Intermediates with biosynthetic implications in *de novo* production of phenyl-phenalenone-type phytoalexins by *Musa acuminata*. Revised structure of emenolone. *Tetrahedron* **1995**, *51*, 4117–4130.
- (22) Kamo, T.; Hirai, N.; Iwami, K.; Fujioka, D.; Ohigashi, H. New phenylphenalenones from banana fruit. *Tetrahedron* **2001**, *57*, 7649–7656.
- (23) Luis, J. G.; Quiñones, W.; Echeverri, F.; Grillo, T. A.; Kishi, M. P.; Garcia, F.-G.; Torres, F.; Cardona, G. Musanolones: four 9-phenylphenalenones from rhizomes of *Musa acuminata*. *Phytochemistry* **1996**, *41*, 753–757.
- (24) Hirai, N.; Ishida, H.; Koshimizu, K. A phenalenone-type phytoalexin from *Musa acuminata*. *Phytochemistry* **1994**, *37*, 383–385.
- (25) Ali, M. S.; Tezuka, Y.; Awale, S.; Banskota, A. H.; Kadota, S. Six new diarylheptanoids from the seeds of *Alpinia blepharocalyx*. *J. Nat. Prod.* **2001**, *64*, 289–293.
- (26) Sasaya, T. Diarylheptanoids of *Alnus hirsuta* Turcz. (Betulaceae). *Enshurin Kenkyu Hokoku (Hokkaido Daigaku Nogakubu)* **1985**, *42*, 191–205; *Chem. Abstr.* **1985**, *103*, 157293r.

Received for review June 14, 2002. Revised manuscript received August 22, 2002. Accepted August 22, 2002. This research was supported by Program Project P01 CA48112, funded by the National Cancer Institute, National Institutes of Health, Bethesda, MD.

JF0206670