Activity-Guided Isolation of Constituents of *Renealmia nicolaioides* with the Potential to Induce the Phase II Enzyme Quinone Reductase

Jian-Qiao Gu,[†] Eun Jung Park,[†] Jose Schunke Vigo,[‡] James G. Graham,[†] Harry H. S. Fong,[†] John M. Pezzuto,[†] and A. Douglas Kinghorn*[,†]

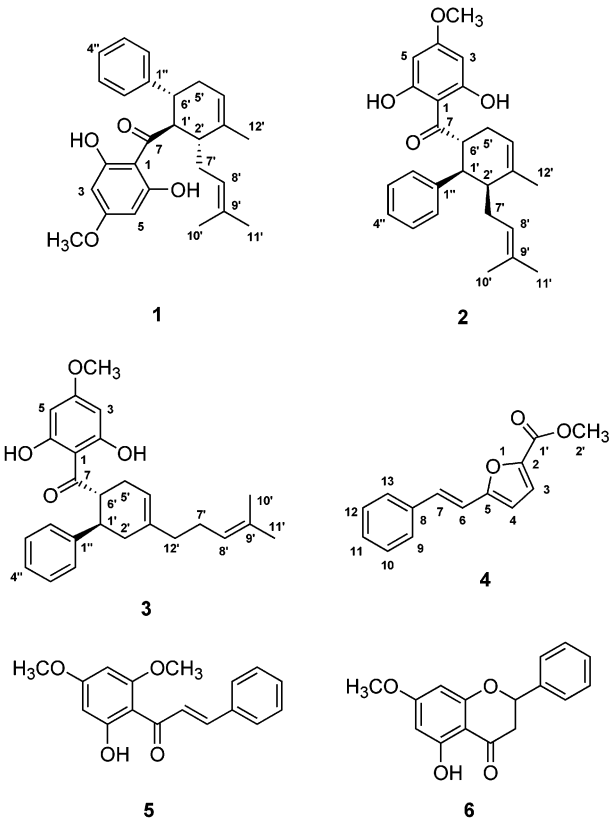
Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, and Instituto Nacional de Medicina Tradicional (INMETRA), Ministerio de Salud, Jesus Maria, Lima, Peru

Received May 31, 2002

Three new prenylated dihydrochalcones, (±)-nicolaioidesins A, B, and C (**1**−**3**), as well as a new natural product, 5-styrylfuran-2-carboxylic acid methyl ester (**4**), along with four known compounds, 2′-hydroxy-4′,6′-dimethoxychalcone (**5**), (±)-5-hydroxy-7-methoxyflavanone (**6**), (±)-5-hydroxy-7,4′-dimethoxyflavanone, and panduratin A, were isolated from the roots of *Renealmia nicolaioides*, using a bioassay to determine the induction of quinone reductase (QR) activity with cultured Hepa lclc7 mouse hepatoma cells. Among these isolates, **5** and **6** induced QR activity, with observed concentrations to double activity (CD) values of 1.7 and 0.9 μg/mL, respectively, while the other constituents were not regarded as being active (CD >10 μg/mL). The chemical structures of **1**−**4** were elucidated by spectroscopic methods. A biogenetic pathway for the formation of (±)-nicolaioidins A−C (**1**−**3**) is proposed.

The genus *Renealmia* comprises about 75 species, of which approximately one-third grow in tropical Africa, while the remainder are found in the Neotropics.[1] Various plant parts of *R. alpinia*, *R. asplundii*, *R. cincinnata*, *R. clomingensis*, *R. exaltata*, and *R. thyrsoidea* have been used traditionally to treat fever,[2] headache,[3] and stomachache.[3] Species in this genus also have medicinal uses as antivenins,[4,5] febrifuges,[6] and tonics,[3] and some are edible.[2,7] Three sesquiterpenoids, 1(10)*E*,5*E*-germacradien-4β-ol, 5*E*,-10(14)-germacradien-1β,4β-diol, and oplodiol, isolated from a methylene dichloride-soluble extract of *R. cincinnata*, were found to exhibit significant in vitro antiplasmodial activity against cultured *Plasmodium falciparum* clone D-6 with IC_{50} values of <5 μg/mL.[2] Previous phytochemical studies with various *Renealmia* species have resulted in the isolation of flavonols,[8] labdane diterpenes,[6,9−11] monoterpenes,[9,12] and eudesmane-, germacrane-, and isodaucane-type sesquiterpenes.[2]

Renealmnia nicolaioides Loes. (Zingiberaceae) is commonly known as "mishqui panga" in the Quechua dialect in Peru, which means "tasty leaf". It is a 2−5 m tall, stout plant that mainly grows in the forests of the western part of tropical South America.[1] No previous biological or phytochemical investigations have been reported on this plant. As part of a continuing collaborative search for novel plant-derived cancer chemopreventive agents,[13,14] a methanolic extract of the roots of *R. nicolaioides* was found to significantly induce quinone reductase (QR) activity with cultured Hepa lclc7 mouse hepatoma cells.[15] Induction of phase II enzymes, such as QR, is considered an important mechanism of protection against tumor initiation, by catalyzing conjugation reactions to detoxify certain procarcinogens or carcinogens.[16,17] In the present investigation, bioassay-guided fractionation of *n*-hexane and ethyl acetate extracts using the QR induction bioassay led to the isolation of three new prenylated dihydrochalcones, nicolaioidesins A, B, and C (**1**−**3**), as well as a new natural product, 5-styrylfuran-2-carboxylic acid methyl ester (**4**), along with four known compounds. The structure elucidation of **1**−**4** and the biological evaluation of all isolates obtained are described herein.



Results and Discussion

Four flavonoids of previously known structure were isolated from the *n*-hexane and ethyl acetate extracts of the roots of *R. nicolaioides*, as described in the Experimental Section, and were identified, in turn, as 2′-hydroxy-4′,6′-dimethoxychalcone (**5**),[18] (±)-5-hydroxy-7-methoxyflavanone (**6**),[19] (±)-5-hydroxy-7,4′-dimethoxyflavanone,[20] and pan-

* To whom correspondence should be addressed. Tel: (312) 996-0914. Fax: (312) 996-7107. E-mail: kinghorn@uic.edu.
[†] University of Illinois at Chicago.
[‡] Instituto Nacional de Medicina Tradicional.

10.1021/np020249p CCC: $22.00
Published on Web 09/20/2002

Table 1. ^{1}H NMR Spectral Data for **1**−**3**[a]

position	1	2	3
3/5	5.71 s	5.92 s	5.84 s
1′	4.38 dd (11, 11)	3.57 dd (5.1, 11)	3.30 ddd (6.1, 11, 11)
2′	2.96 m	2.27 dt (4.9, 5.0)	2.27[b], 2.17[b]
4′	5.61 d (4.4)	5.49 br s	5.51 br s
5′a	2.38 m (15)	2.12 ddd (1.8, 4.2, 18)	2.24[b]
5′b	2.23[b]	2.62 dt (4.2, 18)	2.55 dt (5.2, 17)
6′	3.07 ddd (4.7, 11, 11)	4.76 ddd (6.1, 11, 11)	4.41 ddd (4.8, 11, 11)
7′a	2.17−2.22[b]	1.97 dt-like (15)	2.09[b]
7′b	2.17−2.22[b]	1.87 ddd (5.9, 15)	2.01[b]
8′	5.09 t (6.2)	4.86 t (5.9)	5.11 t (6.8)
10′	1.42 s	1.33 s	1.61 s
11′	1.57 s	1.56 s	1.69 s
12′	1.74 s	1.76 s	2.01[b], 2.09[b]
2″/6″	7.17 d (7.7)	7.08−7.13[b]	7.18−7.26[b]
3″/5″	7.07 t-like (7.7)	7.18 d (7.5)	7.18−7.26[b]
4″	6.98 t-like (7.7)	7.08−7.13[b]	7.09 m (1.8, 7.7)
OCH_3-4	3.70 s	3.76 s	3.75 s

[a] Chemical shifts (ppm) were recorded at 500 MHz using $CDCl_3$ as solvent and TMS as internal standard. Figures in parentheses are coupling constants in Hz. Assignments were based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b]Multiplicity patterns were unclear due to signal overlapping.

Table 2. ^{13}C NMR Spectral Data for **1**−**3**[a]

position	1	2	3
1	107.5 s[b]	105.2 s	107.5 s
2/6	n.d.[c]	162.7 s	n.d.[c]
3/5	95.1 d	94.6 d	94.5 d
4	165.2 s	165.4 s	165.3 s
7	209.5 s	209.2 s	208.5 s
1′	55.1 d	46.0 d	42.7 d
2′	44.4 t	45.6 t	38.2 t
3′	136.1 s	137.6 s	137.6 s
4′	122.8 d	120.1 d	119.2 d
5′	33.9 t	30.9 t	30.7 t
6′	46.7 d	43.8 d	50.1 d
7′	29.2 t	28.0 t	37.3 t
8′	122.1 d	123.8 d	124.2 d
9′	133.8 s	130.4 s	131.6 s
10′	17.9 q	17.8 q	17.7 q
11′	25.7 q	25.9 q	25.7 q
12′	21.4 q	22.9 q	26.4 t
1″	143.2 s	143.4 s	145.2 s
2″/6″	127.9 d	128.1 d	127.3 d
3″/5″	127.8 d	128.1 d	128.3 d
4″	126.3 d	125.7 d	126.0 d
OCH_3-4	55.4 q	55.5 q	55.5 q

[a] Chemical shifts (ppm) were recorded at 125 MHz using $CDCl_3$ as solvent and TMS as internal standard. Assignments were based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b]Signal multiplicity. [c]Signals not detected.

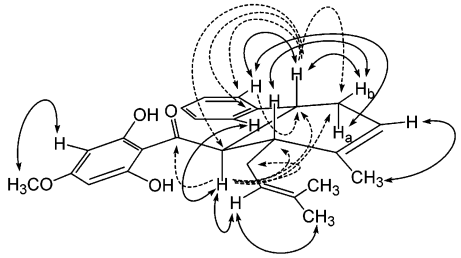


Figure 1. Selected HMBC (dotted line) and NOESY (solid line) correlations for **1**.

duratin A,[21] by comparison of their physical and spectral data with reported values. Compound **6** was the major compound found in the present investigation, in a yield of greater than 0.37% w/w of the dried plant material.

Compound **1**, $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.058, MeOH), was obtained as a white amorphous solid. The molecular formula was determined as $C_{26}H_{30}O_4$ by HREIMS (obsd *m*/*z* 406.2129). This compound exhibited UV maxima at 210, 234, and 296 nm, suggestive of the presence of considerable conjugation in the molecule. In the IR spectrum, absorption bands at 3526−3109 cm^{-1} (hydrogen-bonded OH) and 1625, 1583, and 1520 cm^{-1} (aromatic ring) were apparent. The phenolic nature of the hydroxyl groups was evident from a positive ferric chloride test. The EIMS of **1** exhibited characteristic ion peaks at *m*/*z* 337 (fragment ion due to loss of a prenyl unit at C-2′), 271 (fragment ion due to McLafferty rearrangement, in which the carbonyl group and H-7′ were involved),[22] and 167 (base peak, substituted benzoylium ion).

Comparison of the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **1** with analogous data for the known prenylated dihydrochalcone, panduratin A,[21] which, as mentioned, was also obtained in the present investigation, indicated that they are a pair of diastereomers, with the only difference being in the stereochemistry of the prenyl moiety at C-2′. The stereochemistry of panduratin A was initially determined by various NMR experiments[21] and then further confirmed by single-crystal X-ray diffraction analysis.[23] In the ^{1}H NMR and ^{1}H−^{1}H COSY spectra of **1**, one methine proton at δ_H 4.38 (dd, J = 11, 11 Hz) assigned to H-1′ was observed to couple with protons at δ_H 2.96 (1H, m, H-2′) and 3.07 (1H, ddd, J = 4.7, 11, 11 Hz, H-6′). The coupling constants of 11 Hz for both $J_{H-1'-H-2'}$ and $J_{H-1'-H-6'}$ demonstrated that the two pairs of protons are *trans*-diaxial to each other in a normal half-chair conformation and that the corresponding substituents are consequently disposed equatorially.[24] Moreover, the proposed relative stereochemistry of **1** in the cyclohexene ring was confirmed by the spatial correlations between H-1′ and H-8′ and H-2″/6″, H-2′ and H-5′b, and H-6′ and H-5′b and H-2″/6″, as observed in the NOESY spectrum (Figure 1). The arylacyl group was placed at C-1′ as a result of correlations between H-1′ (δ_H 4.38) and C-7 (δ_C 209.5), C-2′ (δ_C 44.4), C-5′ (δ_C 33.9), C-6′ (δ_C 46.7), and C-7′ (δ_C 29.2) in the HMBC spectrum (Figure 1). Similarly, the phenyl group was located at C-6′ in the cyclohexene ring on the basis of the observed HMBC spectral correlations between H-6′ (δ_H 3.07) and C-1′ (δ_C 55.1), C-5′ (δ_C 33.9), C-1″ (δ_C 143.2), and C-2″/6″ (δ_C 127.9). Compound **1** was determined to be a racemate on the basis of its optical rotation profile, as well as the fact that it exhibited a 1:1 mixture of corresponding esters as determined from its ^{1}H NMR spectrum in $CDCl_3$ after being treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride using Mosher's methodology.[22] Accordingly, the structure of **1** was assigned as the new prenylated dihydrochalcone racemate (1′*R**,2′*R**,6′*R**)-(2,6-dihydroxy-4-methoxyphenyl)-(3′-methyl-6′-phenyl-2′-prenylcyclohex-3′-enyl)methanone, to which the trivial name (±)-nicolaioidesin A has been accorded. The structure shown for **1** is one of the two possible representations of the relative stereochemistry. Although several prenylated dihydrochalcones with an arylacyl unit at C-1′ and a phenyl group at C-6′ were reported previously,[21,22,25] compound **1** serves as the first representative in this series in which H-1′ and H-2′ exhibit a *trans*-diaxial relationship.

Compound **2**, $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.11, MeOH), was obtained as a pale yellowish amorphous solid. The molecular formula was determined as $C_{26}H_{30}O_4$ by HREIMS (obsd *m*/*z* 406.2127). As documented in the Experimental Section, **2** also exhibited UV, IR, ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), and mass spectral features similar to those of panduratin A[21] and was therefore recognized as being a further positional isomer of the latter compound, with the differences occurring in the respective placement of the aryl-

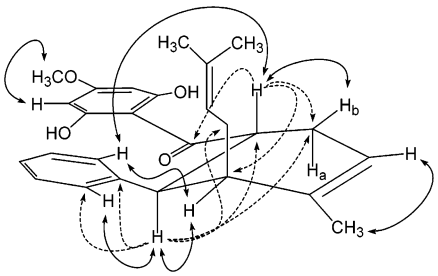


Figure 2. Selected HMBC (dotted line) and NOESY (solid line) correlations for **2**.

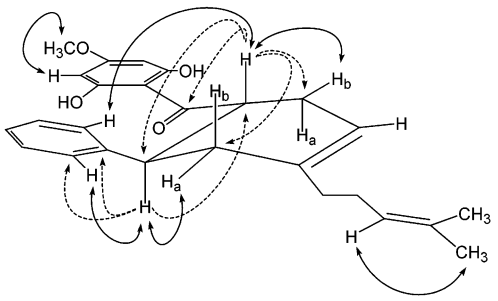


Figure 3. Selected HMBC (dotted line) and NOESY (solid line) correlations for **3**.

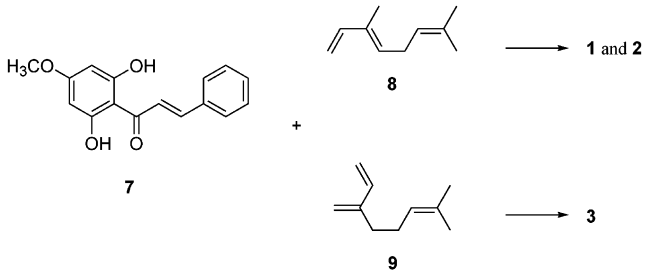


Figure 4. Biogenetic proposal for the formation of (±)-nicolaioidins A−C (**1**−**3**).

acyl and phenyl groups at C-1′ and C-6′. A significant difference between **2** and panduratin A was observed in the aliphatic region of their high-field ^{1}H NMR spectra. Signals due to H-1′ (δ_H 3.57, dd, J = 5.1, 11 Hz) and H-6′ (δ_H 4.76, ddd, J = 6.1, 11, 11 Hz) in **2** were assigned by cross-peaks in the ^{1}H−^{1}H COSY spectrum and their similar splitting patterns, when compared with H-1′ (δ_H 4.78, dd, J = 4.4, 11 Hz) and H-6′ (δ_H 3.45, ddd, J = 6.3, 11, 11 Hz) in panduratin A.[21] Moreover, the arylacyl group was placed at C-6′ in **2** as a result of correlations between H-6′ (δ_H 4.76) and C-7 (δ_C 209.2), C-2′ (δ_C 45.6), and C-5′ (δ_C 30.9) in the HMBC spectrum (Figure 2), and the phenyl group at C-1′ on the basis of the observed HMBC cross-peaks for H-1′ (δ_H 3.57) and C-5′ (δ_C 30.9), C-6′ (δ_C 43.8), C-7′ (δ_C 28.0), C-1″ (δ_C 143.4), and C-2″/6″ (δ_C 128.1). Furthermore, the coupling constants of 5.1 Hz for $J_{H-1'-H-2'}$ and 11 Hz for $J_{H-1'-H-6'}$ observed in the ^{1}H NMR spectrum of **2** demonstrated that H-1′ and H-2′ must be *cis*-oriented, while H-1′ and H-6′ are *trans*-diaxial to each other in a normal half-chair conformation.[21] The proposed relative stereochemistry of **2** was confirmed by the spatial correlations between H-1′ and H-2′ and H-2″/6″, H-2′ and H-1′ and H-2″/6″, and H-6′ and H-5′b and H-2″/6″, as observed in the NOESY spectrum (Figure 2). Compound **2** was also determined as a racemate using the above-mentioned techniques for **1**. Therefore, the structure of **2** was assigned as (1′*R**,2′*S**,6′*R**)-(2,6-dihydroxy-4-methoxyphenyl)-(3′-methyl-1′-phenyl-2′-prenylcyclohex-3′-enyl)methanone, to which the trivial name (±)-nicolaioidesin B has been accorded. The structure shown for **2** is one of the two possible representations of the relative stereochemistry.

Compound **3**, $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.042, MeOH), was obtained as a white amorphous solid. The molecular formula was also determined as $C_{26}H_{30}O_4$ by HREIMS (obsd *m*/*z* 406.2129). Compound **3** was recognized as an isomer of **2** on the basis of their quite similar spectroscopic data, as described in the Experimental Section. Comparison of the ^{1}H NMR spectral data (Table 1) of **3** with those of **2** indicated that **3** exhibited two additional methylene protons, δ_H 2.17 and 2.27 for H_2-2′ and δ_H 2.24 and 2.55 for H_2-5′, instead of a methine proton signal at δ_H 2.27 (dt, J = 4.9, 5.1 Hz, H-2′) and a methyl signal at δ_H 1.76 (s, H_3-12′) in **2**, while their remaining proton signals were closely comparable. Moreover, these differences were also confirmed by their ^{13}C NMR spectral data, as shown in Table 2, where **3** exhibited two additional secondary carbons at δ_C 38.2 (C-2′) and 26.4 (C-12′), instead of the tertiary carbon at δ_C 45.6 (C-2′) and the primary carbon at δ_C 22.9 (C-12′) in **2**. In an analogous manner to compound **2**, the arylacyl group was placed at C-6′ in **3** as a result of correlations between H-6′ (δ_H 4.41) and C-7 (δ_C 208.5), C-1′ (δ_C 42.7), C-2′ (δ_C 38.2), and C-5′ (δ_C 30.7) in the HMBC spectrum (Figure 3), while the phenyl group was positioned at C-1′ on the basis of the observed HMBC cross-peaks for H-1′ (δ_H 3.30) and C-6′ (δ_C 50.1), C-1″ (δ_C 145.2), and C-2″/6″ (δ_C 127.3). Furthermore, the coupling constant of 11 Hz for H-1′ and H-6′ indicated that they are *trans*-axial to each other in a normal half-chair conformation.[21] The proposed relative stereochemistry of **3** was further confirmed by the spatial correlations between H-1′ and H-2′a and H-2″/6″, and H-6′ and H-5′b and H-2″/6″, as observed in the NOESY spectrum (Figure 3). Compound **3** was also determined as a racemate using the same techniques described for **1**. Accordingly, the structure of **3** was assigned as (1′*R**,6′*R**)-(2,6-dihydroxy-4-methoxyphenyl)-(3′-isohexenyl-1′-phenylcyclohex-3′-enyl)-methanone and has been named (±)-nicolaioidesin C. The structure shown for **3** is one of the two possible representations of the relative stereochemistry.

Biogenetically, (±)-nicolaioidesins A−C (**1**−**3**) can be regarded as products resulting from Diels−Alder-like cyclization of 2′,6′-dihydroxy-4′-methoxychalcone (**7**) and the acylic monoterpene *β*-ocimene (**8**) or myrcene (**9**) (Figure 4).[26,27] The fact that all of the isolated nicolaioidesins were obtained as racemates indicates that the initial [2+4] cycloaddition reaction in the proposed biosynthesis is not enantioselective.

Compound **4** was obtained as a white amorphous powder. The molecular formula was also determined as $C_{14}H_{12}O_3$ by HREIMS (obsd *m*/*z* 228.0783). This compound exhibited UV maxima at 210, 231, 255, and 343 nm, suggesting the presence of considerable conjugation in the molecule. In the IR spectrum, absorption bands at 1719 (α,*β*-unsaturated ester) and 1637 and 1557 cm^{-1} (aromatic ring) were apparent. Its ^{1}H NMR spectrum showed four isolated spin systems, inclusive of one methoxy group at δ_H 3.83 (3H, s), two pairs of coupled vinylic protons at δ_H 5.50 and 5.95 (both 1H, d, J = 2.2 Hz) and δ_H 6.59 and 7.51 (both 1H, d, J = 16 Hz), and one monosubstituted benzene ring at δ_H 7.34−7.40 (3H, m) and δ_H 7.49−7.52 (2H, m). A furan ring moiety in **4** was deduced from the molecular formula and the calculated degrees of unsaturation. The ester group was placed at C-2 rather than C-4 in **4** as a result of HMBC cross-peaks observed between H-6 and C-4, C-5, and C-8. The stereochemistry of the double bond between C-6 and C-7 was determined as being in the *E*-configuration on the basis of the observed coupling constant of 16 Hz for H-6

and H-7. Accordingly, compound **4**, a new natural product, was assigned as 5-styrylfuran-2-carboxylic acid methyl ester. This compound was previously synthesized,[28] but no NMR data were reported.

All of the isolates obtained from the roots of *R. nicolaioides* were evaluated for their potential to induce quinone reductase (QR) activity in murine Hepa 1c1c7 hepatoma cells.[29] The results showed that **5** and **6** induced QR activity, with observed concentrations to double induction (CD) values of 1.7 and 0.9 μg/mL, respectively, while the other constituents were not regarded as active (CD >10 μg/mL). Compounds **5** and **6** were further tested for their cytotoxicity for Hepa 1c1c7 cells and exhibited IC_{50} values of 7.2 and >100 μg/mL, respectively. A superior chemopreventive index (CI = IC_{50}/CD) value for **6** (>111) was evident, when compared with the CI value of 23.8 for sulforaphane (CD, 0.087 μg/mL; IC_{50}, 2.07 μg/mL), a positive control used for this assay.[29,30] Therefore, (±)-5-hydroxy-7-methoxyflavanone (**6**) may be considered a promising lead for further evaluation as a cancer chemopreventive agent.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were obtained with an ATI Mattson FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer. HREIMS and LREIMS were recorded on a Finnigan MAT 95 mass spectrometer (70 eV). HPLC was performed on a Waters system with two 515 pumps and a 996 photodiode array detector, using 20 × 250 mm, 5 μm YMC-Pack ODC-AQ and CN columns (YMC Co., Ltd., Wilmington, NC) at 9 mL/min and monitored at 290 and 343 nm. Compounds were visualized on TLC plates by dipping in 10% $FeCl_3$ in EtOH, along with the use of phosphomolybdic acid reagent (Aldrich, Milwaukee, WI) followed by charring at 110 °C for 5−10 min.

Plant Material. The roots of *R. nicolaioides* were collected at Tocache, San Martin, Peru, in September 1999 by J.S.V. and J.G.G. A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. P-03417).

Biological Assays for the Induction of Quinone Reductase (QR) with Cultured Mouse Hepatoma Cells. For the evaluation of plants extracts, fractions, and pure isolates as inducers of QR, cultured mouse Hepa 1c1c7 cells were used as described previously.[29,30] Enzyme activity was expressed as CD, the concentration required to double the specific activity of QR. IC_{50} (half-maximal inhibitory concentration of cell viability) and CI (chemoprevention index, IC_{50}/CD) values were also determined.

Extraction and Isolation. The milled plant material (500 g) was extracted by maceration with MeOH (3 L × 3). After filtration and evaporation of the solvent in vacuo, the resultant extract was diluted with H_2O to afford an aqueous MeOH solution (90%) and then partitioned with *n*-hexane and EtOAc, respectively, to afford dried *n*-hexane-soluble (3.0 g) and EtOAc-soluble (5.5 g) residues. Both the *n*-hexane and the EtOAc extracts, which significantly induced QR activity with CD values of 3.2 and 6.1 μg/mL, respectively, were subjected to Si gel column chromatography by elution with increasing concentrations of MeOH in $CHCl_3$ to give six (F01−F06) and five (F07−F11) fractions, respectively. Fractions F01, F02, F07, and F08, which were all active in the QR assay (CD <10 μg/mL), were combined on the basis of their similar TLC profiles (SiO_2, $CHCl_3$−MeOH, 50:1 to 20:1). Chromatography of the combined fractions (5.4 g) over Si gel by elution with gradient mixtures of *n*-hexane−acetone (20:1 to 2:1) gave seven further fractions (F13−F19). Fraction 13 (2.1 g, CD value of <2.5 μg/mL) was further fractionated (SiO_2, stepwise, *n*-hexane−EtOAc, 50:1 to 15:1) to afford (±)-5-hydroxy-7-methoxyflavanone[19] (**6**, 1.87 g) and a subfraction (74 mg, *n*-hexane−EtOAc, 15:1), which was purified by reversed-phase HPLC (YMC-Pack ODC-AQ column, CH_3CN−H_2O, 6.12:2.88) to afford (±)-5-hydroxy-7,4′-dimethoxyflavanone (5.5 mg, t_R 9.09 min),[20] (±)-5-hydroxy-7-methoxyflavanone (**6**, 12 mg, t_R 9.68 min),[19] and 2-hydroxy-4,6-dimethoxychalcone (**5**, 23 mg, t_R 14.9 min).[18] Fraction 15 (260 mg, CD value of 4.3 μg/mL) was further fractionated by reversed-phase HPLC (YMC-Pack ODC-AQ column, CH_3CN−H_2O, 7.38:1.62) to afford **4**[26] (16 mg, t_R 7.3 min) and two subfractions i and ii (140 mg, t_R 18.2 min; 10 mg, t_R 22.8 min, respectively). Panduratin A (42 mg),[21] **1** (2.2 mg), and **2** (15 mg) were obtained from subfraction i by HPLC (YMC-Pack CN column, *n*-hexane−2-propanol−methanol, 8.46:0.18:0.18; t_R 16.9, 18.7, and 24.6 min, respectively). Similarly, **3** (1.0 mg) was purified from subfraction ii by HPLC (YMC-Pack CN column, *n*-hexane−2-propanol−methanol, 8.46:0.18:0.18; t_R 19.8 min).

(1′*R,2′*R**,6′*R**)-(2,6-Dihydroxy-4-methoxyphenyl)-(3′-methyl-6′-phenyl-2′-prenylcyclohex-3′-enyl)methanone [(±)-nicolaioidesin A, 1]:** white amorphous solid; $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.058, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.47), 234 (3.96), 296 (4.08) nm; IR (dried film) ν_{max} 3526−3109, 1625, 1583, 1520, 1425, 1372, 1227, 1210, 1166 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* $[M]^+$ 406 (3), 337 (5), 271 (4), 233 (2), 167 (100); HREIMS *m/z* 406.2129 $[M]^+$ (calcd for $C_{26}H_{30}O_4$, 406.2144).

(1′*R,2′*S**,6′*R**)-(2,6-Dihydroxy-4-methoxyphenyl)-(3′-methyl-1′-phenyl-2′-prenylcyclohex-3′-enyl)methanone [(±)-nicolaioidesin B, 2]:** pale yellowish amorphous solid; $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.45), 231 (4.00), 289 (4.13) nm; IR (dried film) ν_{max} 3610−3107, 1628, 1584, 1520, 1449, 1427, 1378, 1228, 1162 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* $[M]^+$ 406 (2), 388 (0.5), 337 (0.8), 271 (2), 167 (100); HREIMS *m/z* 406.2127 $[M]^+$ (calcd for $C_{26}H_{30}O_4$, 406.2144).

(1′*R,6′*R**)-(2,6-Dihydroxy-4-methoxyphenyl)-(3′-isohexenyl-1′-phenylcyclohex-3′-enyl)methanone [(±)-nicolaioidesin C, 3]:** white amorphous solid; $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.042, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.69), 229 (4.10), 290 (4.24) nm; IR (dried film) ν_{max} 3546−3167, 1624, 1583, 1520, 1426, 1375, 1240, 1208, 1165 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m/z* $[M]^+$ 406 (2), 337 (2), 271 (2), 238 (2), 167 (100); HREIMS *m/z* 406.2129 $[M]^+$ (calcd for $C_{26}H_{30}O_4$, 406.2144).

5-Styrylfuran-2-carboxylic acid methyl ester (4): white powder; mp 130−132 °C (lit.[15] 119−120 °C); UV (MeOH) λ_{max} (log ϵ) 210 (4.32), 231 (4.02), 255 (3.96), 343 (4.18) nm; IR (dried film) ν_{max} 1719, 1696, 1637, 1557, 1456, 1407, 1255, 1153 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.83 (3H, s, H-2′), 5.50 (1H, d, J = 2.2 Hz, H-3), 5.95 (1H, d, J = 2.2 Hz, H-4), 6.59 (1H, d, J = 16 Hz, H-6), 7.34−7.40 (3H, overlapped), 7.49−7.52 (3H, overlapped); ^{13}C NMR ($CDCl_3$) δ 56.0 (q, C-2′), 88.9 (d, C-3), 101.4 (d, C-4), 118.6 (d, C-6), 127.5 (d, C-9 and 13), 128.9 (d, C-10 and 12), 129.5 (d, C-11), 135.2 (s, C-8), 135.8 (d, C-7), 158.6 (s, C-5), 164.0 (s, C-2), 171.0 (s, C-1′); EIMS *m/z* $[M]^+$ 228 (100), 200 (35), 185 (11), 157 (31), 149 (21), 129 (15), 103 (10); HREIMS *m/z* 228.0783 $[M]^+$ (calcd for $C_{14}H_{12}O_3$, 228.0786).

2′-Hydroxy-4′,6′-dimethoxychalcone (5): yellowish powder; mp 80−82 °C (lit.[31] 84−85 °C); UV, IR, ^1H NMR, and ^{13}C NMR data, consistent with literature values.[18,31]

(±)-5-Hydroxy-7-methoxyflavanone (6): pale yellowish prisms ($CHCl_3$−MeOH); mp 85−87 °C (lit.[19] 84−85 °C); $[\alpha]^{20}_{\lambda}$ 0° (*c* 0.18, MeOH); CD nm (MeOH) $\Delta\epsilon_{290}$ 0; UV, IR, ^1H NMR, and ^{13}C NMR data, consistent with literature values.[19]

Acknowledgment. This research was supported by program project P01 CA48112, funded by the National Cancer Institute, NIH, Bethesda, MD. We thank Dr. Rob Kleps of the Research Resources Center, University of Illinois at Chicago, for facilitating the running of the 500 MHz NMR spectra, and Dr. Keith Fagerquist of the Mass Spectrometry Service Laboratory, Department of Chemistry, University of Minnesota, for recording the EIMS data.

Supporting Information Available: List of physical and spectral data for the known compounds (±)-5-hydroxy-7,4′-dimethoxyflavanone and panduratin A. This material is available free of charge via the Internet at http://www.pubs.acs.org.

References and Notes

(1) Mass, P. J. M. *Flora Neotropica: Monograph No. 18. Renealmia (Zingiberaceae−Zingiberoideae) and Costoideae (Additions)*; The New York Botanical Garden: Bronx, NY, 1977; pp 100−103.
(2) Chuendem, M. H.; Mbah, J. A.; Tsopmo, A.; Ayafor, J. F.; Sterner, O.; Okunjic, C. C.; Iwu, M. M.; Schuster, B. M. *Phytochemistry* **1999**, *52*, 1095−1099.
(3) Milliken, W.; Albert, B. *Econ. Bot.* **1996**, *50*, 10−25.
(4) Selvanayagam, Z. E.; Gnanavendhan, S. G.; Balakrishna, K.; Rao, R. B. *J. Herbs Species Med. Plants* **1994**, *2*, 45−100.
(5) Davis, E. W.; Yost, J. A. *J. Ethnopharmacol.* **1983**, *9*, 273−297.
(6) Zhou, B. N.; Baj, N. J.; Glass, T. E.; Malone, S.; Werkhoven, M. C.; van Troon, F.; Wisse, J. H.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1287−1293.
(7) Lentz, D. L. *Econ. Bot.* **1993**, *47*, 358−370.
(8) Williams, C. A.; Harborne, J. B. *Biochem. Syst. Ecol.* **1977**, *5*, 221−229.
(9) Lognay, G.; Marlier, M.; Severin, M.; Haubruge, E.; Gibon, V.; Trevejo, E. *Flavour Fragrance J.* **1991**, *6*, 87−91.
(10) Ramiandrasoa, F.; Chuilon, S.; Moretti, C.; Kunesch, G. *Plant Med. Phytother.* **1986**, *20*, 227−230.
(11) Yang, S. W.; Zhou, B. N.; Malone, S.; Werkhoven, M. C.; van Troon, F.; Wisse, J. H.; Kingston, D. G. I. *J. Nat. Prod.* **1999**, *62*, 1173−1174.
(12) Luz, A. I. R.; Zoghbi, M. G. B.; Ramos, L. S.; Maia, J. G. S.; Silva, M. L. *J. Nat. Prod.* **1984**, *47*, 907−908.
(13) Pezzuto, J. M. *Biochem. Pharmacol.* **1997**, *53*, 121−133.
(14) Kinghorn, A. D.; Fong, H. H. S.; Farnsworth, N. R.; Mehta, R. G.; Moon, R. C.; Moriarty, R. M.; Pezzuto, J. M. *Curr. Org. Chem.* **1998**, *2*, 597−612.
(15) Prochaska, H. J.; Santamaria, A. B. *Anal. Biochem.* **1988**, *169*, 328−336.
(16) Zhang, Y.; Talalay, P.; Cho, C. G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399−2403.
(17) Gerhäuser, C.; You, M.; Liu, J.; Moriarty, R. M.; Hawthorne, M.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Cancer Res.* **1997**, *57*, 272−278.
(18) Patra, A.; Ghosh, G.; Sengupta, P. K.; Nath, S. *Magn. Reson. Chem.* **1987**, *25*, 734−736.
(19) Duddeck, H.; Snatzke, G.; Yemul, S. S. *Phytochemistry* **1978**, *17*, 1369−1373.
(20) Burke, B.; Nair, M. *Phytochemistry* **1986**, *25*, 1427−1430.
(21) Tuntiwachwuttikul, P.; Pancharoen, O.; Reutrakul, V.; Byrne, L. T. *Aust. J. Chem.* **1984**, *37*, 449−453.
(22) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4095.
(23) Pancharoen, O.; Reutrakul, V.; Skelton, B. W.; Taylor, W. C.; Tuntiwachwuttikul, P.; White, A. H. *Aust. J. Chem.* **1984**, *37*, 2589−2592.
(24) Nomura, T.; Fukai, T.; Hano, Y.; Uzawa, J. *Heterocycles* **1982**, *17*, 381−389.
(25) Trakoontivakorn, G.; Nakahara, K.; Shinmoto, H.; Takenaka, M.; Onishi-Kameyama, M.; Ono, H.; Yoshida, M.; Nagata, T.; Tsushida, T. *J. Agric. Food Chem.* **2001**, *49*, 3046−3050.
(26) Nkunya, M. H. H.; Achenbach, H.; Renner, C.; Waibel, R.; Weenen, H. *Phytochemistry* **1990**, *29*, 1261−1264.
(27) Shibata, K.; Tatsukawa, A.; Umeoka, K.; Lee, H. S.; Ochi, M. *Tetrahedron* **2000**, *56*, 8821−8824.
(28) Yoshina, S.; Tanaka, A.; Yang, G. N. *Yakugaku Zasshi* **1968**, *88*, 977−983.
(29) Misico, R. I.; Song, L. L.; Veleiro, A. S.; Cirigliano, A. M.; Tettamanzi, M. C.; Burton, G.; Bonetto, G. M.; Nicotra, V. E.; Silva, G. L.; Gil, R. R.; Oberti, J. C.; Kinghorn, A. D.; Pezzuto, J. M. *J. Nat. Prod.* **2002**, *65*, 677−680.
(30) Chang, L. C.; Gerhäuser, C.; Song, L.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1997**, *60*, 869−873.
(31) Rao, C. B.; Rao, T. N.; Suryaprakasam, S. *Planta Med.* **1976**, *29*, 391−392.

NP020249P