New Chemical Constituents of *Euphorbia quinquecostata* and Absolute Configuration Assignment by a Convenient Mosher Ester Procedure Carried Out in NMR Tubes

Bao-Ning Su,[†] Eun Jung Park,[†] Zakaria H. Mbwambo,[‡] Bernard D. Santarsiero,[§] Andrew D. Mesecar,[§] 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, Chicago, Illinois 60612, Institute of Traditional Medicine, Muhimbili University College of Health Sciences, Dar-es-Salaam, Tanzania, and Center for Pharmaceutical Biotechnology, College of Pharmacy, University of Illinois at Chicago, Illinois 60607

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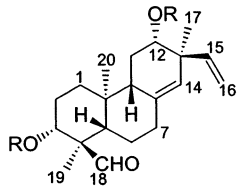
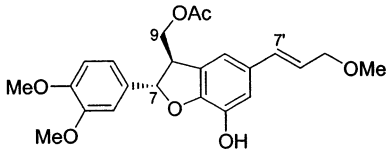
Two new compounds, an *ent*-isopimarane-type diterpene, 3α,12α-dihydroxy-*ent*-8(14),15-isopimaradien-18-al (**1**), and a dihydrobenzo[*b*]furan neolignan, (−)-*trans*-9-acetyl-4,9′-di-*O*-methyl-3′-de-*O*-methyldehydrodiconiferyl alcohol (**2**), along with five known compounds, 7,7′-dihydroxy-6,8′-bicoumarin (bicoumol) (**3**), 3,4-dimethoxycinnamaldehyde (**4**), 6-hydroxy-7-methoxycoumarin (isoscopoletin), *N*-butylaniline, and vanillin, have been isolated from an ethyl acetate-soluble extract of the stem wood of *Euphorbia quinquecostata*. The structures of compounds **1** and **2** were elucidated on the basis of spectroscopic data interpretation, and single-crystal X-ray diffraction analysis was used to confirm the structure and relative stereochemistry of **1**. The absolute configuration of **1** was established by a convenient Mosher ester procedure in which the sample was treated with MTPA chlorides in deuterated pyridine directly in NMR tubes. All isolates were evaluated for the induction of quinone reductase in Hepa1c1c7 hepatoma cells and for the inhibition of the transformation of murine epidermal JB6 cells.

Several phorbol dibutyrate receptor-binding (PDBu) inhibitory diterpenoids of the *ent*-atisane and ingenane classes have been isolated from *Euphorbia quinquecostata* Volk. (Euphorbiaceae) in our previous study.[1] As part of a project directed toward the search for novel, plant-derived cancer chemopreventive agents,[2−4] we have reinvestigated the chemical constituents of an EtOAc-soluble extract of a re-collection of *E. quinquecostata*, with the intention of obtaining a larger quantity of the compound 17-hydroxyingenol 20-hexadecanoate for additional biological testing. During this study, two new compounds, 3α,12α-dihydroxy-*ent*-8(14),15-isopimaradien-18-al (**1**) and (−)-*trans*-9-acetyl-4,9′-di-*O*-methyl-3′-de-*O*-methyldehydrodiconiferyl alcohol (**2**), along with five known compounds, were isolated and characterized. All isolates were evaluated for their potential cancer chemopreventive properties utilizing in vitro assays to determine quinone reductase induction in murine Hepa1c1c7 hepatoma cells[5] and the inhibition of the transformation of murine epidermal JB6 cells.[6] This article reports the isolation and structure elucidation of compounds **1** and **2**, as well as the biological evaluation of all isolates obtained in these two assays. In addition, we wish to present a convenient procedure for determining the absolute configuration of natural products possessing secondary hydroxyl groups, as performed on **1** using the Mosher ester method carried out directly in NMR tubes. It is anticipated that this procedure will have wide applicability to other natural product laboratories, since the reaction conditions have been simplified and very small sample amounts are needed.



1 R = H
1r R = (*R*)-MTPA
1s R = (*S*)-MTPA



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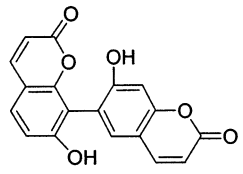

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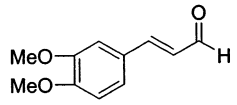

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Results and Discussion

Compound **1** was isolated as colorless needles ($CHCl_3$−MeOH, 1:1), mp 149−150 °C, $[\alpha]_D^{23}$ +7.0° (*c* 0.50, $CHCl_3$).

* To whom correspondence should be addressed. Tel: +1-312-996-0914. Fax: +1-312-996-7107. E-mail: kinghorn@uic.edu.
[†] Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois at Chicago.
[‡] Institute of Traditional Medicine, Muhimbili University.
[§] Center for Pharmaceutical Biotechnology, University of Illinois at Chicago.

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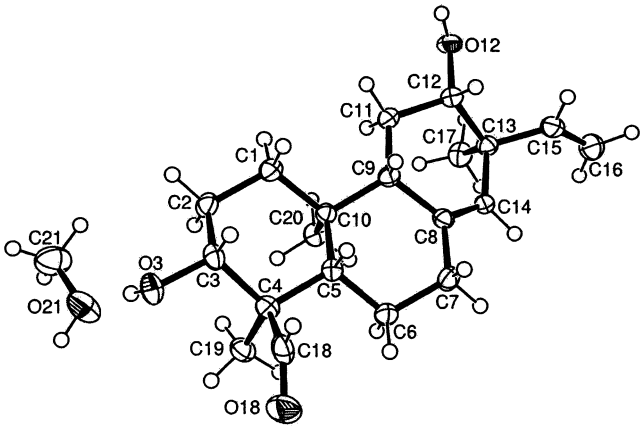

Figure 1. ORTEP drawing of compound **1**.

A molecular formula of $C_{20}H_{30}O_3$ was determined for **1** from its HREIMS at *m/z* 318.2183 (calcd for $C_{20}H_{30}O_3$, 318.2195). The ^{1}H NMR spectrum of compound **1** displayed characteristic signals for an aldehyde proton at δ_H 9.41 (1H, s, H-18), four olefinic protons at δ_H 5.76 (1H, dd, J = 17.5, 10.7 Hz, H-15) and 5.09−5.17 (3H, overlapped, H-16a, H-16b, and H-14), two oxygenated methine protons at δ_H 3.81 (1H, dd, J = 11.6, 4.1 Hz, H-3) and 3.57 (1H, dd, J = 12.2, 4.1 Hz, H-12), and three tertiary methyl singlets at δ_H 1.11 (3H, s, CH_3-19), 1.06 (3H, s, CH_3-17), and 0.87 (3H, s, CH_3-20). The ^{13}C and DEPT NMR spectral data indicated that compound **1** contains 20 carbons, including three methyls, six methylenes, seven methines, and four quaternary carbons. An olefinic proton (δ_H 5.76, H-15) of **1** appeared as a double doublet with coupling constants of 17.6 and 10.5 Hz. These coupling constants and the chemical shift (δ_C 145.9, d, assigned by HMQC correlation) of C-15 suggested the presence of a vinyl group in the molecule of **1**.[6,7] On the basis of the molecular formula determined by HREIMS and from the three unsaturated units (an aldehyde group and two double bonds) inferred from the NMR data, it was apparent that three rings were present in the molecule of **1**. All of this evidence was suggestive that compound **1** is a pimarane-type diterpene.[6,8,9] The positions of the two hydroxy groups and the aldehyde group, as well as the double bond between C-8 and C-14 in **1**, were determined on the basis of the observed HMBC correlations from H-3 to C-18, C-19, C-5, and C-4, from H-12 to C-15, C-17, C-13, and C-14, from H-15 to C-12, C-13, C-14, and C-17, and from CH_3-19 to C-18, C-3, C-4, and C-5. In the NOESY spectrum of **1**, H-18 correlated to H-3 and H-5, CH_3-19 correlated to CH_3-20, and H-12 correlated to H-9 and H-15. These observations indicated that both OH-3 and OH-12 adopt an α-orientation, while the aldehyde and vinyl groups are β-oriented. A suitable crystal was obtained from $CHCl_3$−MeOH (∼1:1), and the X-ray diffraction structure of compound **1** (Figure 1) confirmed its structure and relative configuration.

The Mosher ester procedure[10,11] and modified Mosher method[12,13] are based on the diamagnetic effect of an introduced phenyl ring to assign the absolute stereochemistry of organic compounds by measuring and comparing the NMR data of the resultant diastereomeric α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters. The MTPA ester derivatives obtained are suitable for this purpose because they are thermally stable, do not undergo racemization, and can be readily analyzed by ^{1}H NMR spectroscopy.[14] Particularly as a result of the widespread availability of high-resolution NMR instruments, the absolute configurations of many natural products have been

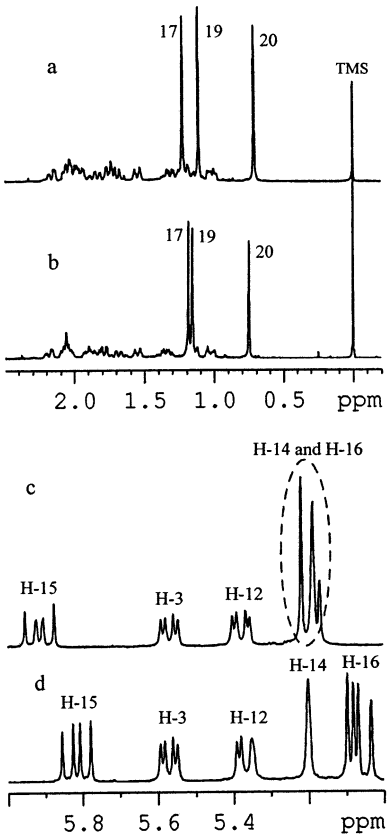

Figure 2. Partial ^{1}H NMR spectra of (*R*)-and (*S*)-MTPA esters (**1r** and **1s**) of compound **1**. The chemical shifts of undisturbed signals of CH_3-17, CH_3-19, and CH_3-20 of **1s** (a) and **1r** (b) are clearly different (TMS was used as internal standard for both), and the vinyl group signals (H-15 and H_2-16) of **1s** (c) showed evident downfield shifts when compared to those of **1r** (d).

successfully established by the widely used MTPA Mosher ester method, e.g., refs 15−21. However, some natural products can be obtained only in very small amounts, and the sample quantities available for chemical transformation and absolute configuration determination for these compounds may become depleted after measurement of the necessary physical data and evaluation of biological activity. In the present study, we have determined the absolute configuration of **1** using a very convenient Mosher ester procedure carried out in NMR tubes. Two portions (each 2.0 mg) of **1** were treated with (*S*)-(+)-α- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) in deuterated pyridine (0.5 mL) directly in separate NMR tubes (Experimental Section) at room temperature, which afforded the (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**, respectively) of **1**. The ^{1}H NMR spectra of **1r** and **1s** were obtained by measuring the reaction NMR tubes directly. [Partial ^{1}H NMR spectra in the diagnostic regions are shown in Figure 2, while the entire ^{1}H NMR spectra of the (*R*)- and (*S*)-MTPA esters of **1** are shown in the Supporting Information.] Although strong proton signals of the excess MTPA chlorides and MTPA acids (hydrolysis products from MTPA chlorides, due to the trace amount of H_2O in deuterated pyridine and the moisture of the experimental environment) were present in the ^{1}H NMR spectra of **1r** and **1s** (Supporting Information), the undisturbed signals (H-15, H-14, H_2-16, CH_3-17, CH_3-19, and CH_3-20) of the diastereomeric MTPA esters (**1r** and **1s**) were clearly different (Figure 2) and the observed chemical shift differences ($\Delta\delta_{S-R}$, Figure 3) unambiguously indicated the absolute configurations of C-3 and C-12 of **1** to be *R*

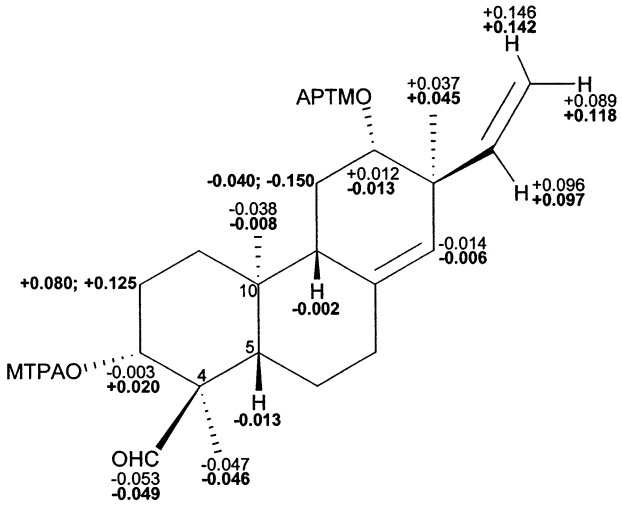

Figure 3. Values of $\delta_S-\delta_R$ (normal and bold data obtained in pyridine-d_5 and $CDCl_3$, respectively) of the MTPA esters of **1**.

and *S*, respectively. Thus, the structure of compound **1** was assigned as 3α,12α-dihydroxy-*ent*-8(14),15-isopimaradien-18-al. This assignment was consistent with the relative configurations of C-3 and C-12 determined from the X-ray analysis of **1**.

To evaluate the reliability of the assigned absolute stereochemistry of **1** by this convenient Mosher ester reaction carried out in NMR tubes, both **1r** and **1s** were individually purified chromatographically. The ^{1}H NMR data of **1r** and **1s** after purification (summarized in Figure 3) also suggested the absolute configuration of C-3 and C-12 of **1** to be *R* and *S*, respectively. The only difference in the present procedure from previously reported Mosher ester methods is that deuterated pyridine and NMR tubes have been used as the reaction solvent and the reaction containers, respectively. In this manner, it is very convenient to monitor reactions by acquiring ^{1}H NMR spectra at intervals, and the absolute stereochemistry can be established from the ^{1}H NMR data of the diastereomeric MTPA esters without further purification. In addition to compound **1**, we have shown the applicability of this convenient procedure to the absolute stereochemistry determination of another secondary hydroxyl-containing natural product, philadelphicalactone A, which was isolated from *Physalis philadelphica* in our recent work, and its absolute configuration has been established by the normal Mosher ester method.[22] The obtained ^{1}H NMR spectral data of (*R*)- and (*S*)-MTPA esters of philadelphicalactone A by the present convenient method (Supporting Information) enabled the absolute configuration of C-4 of philadelphicalactone A to be determined as *S*, which is consistent with the previous result.[22] We believe this convenient procedure will prove to be of wide application in natural products laboratories and will permit the accurate absolute configuration assignment of minor isolates obtained in phytochemical investigations in a time-saving manner.

Compound **2** was isolated as yellowish oil, $[\alpha]_D^{23}$ −73.3° (*c* 0.10, $CHCl_3$). The HREIMS of **2** exhibited a molecular ion peak at *m*/*z* 414.1689, consistent with the molecular formula $C_{23}H_{26}O_7$ (calcd for $C_{23}H_{26}O_7$, 414.1679). In the ^{1}H NMR spectrum of **2**, signals were evident for five aromatic protons (δ_H 6.88−6.91, 5H, m), protons of a *trans* double bond (δ_H 6.56, 1H, d, J = 15.8 Hz, H-7′; 6.16, 1H, dt, J = 15.8, 6.0 Hz, H-8′), three methoxy group protons (δ_H 3.91, 3H, s, Ar−OMe; 3.88, 3H, s, Ar−OMe; 3.40, 3H, s, OMe-9′), and the methyl protons (δ_H 2.04, 3H, s, OAc) of an acetoxyl group. The ^{13}C NMR spectrum of **2** also displayed the characteristic signals for three methoxy groups (δ_C 56.0, Ar−OMe × 2, and 50.3, OMe-9′) and an acetoxyl group (δ_C 170.8, and 20.8). Besides these substituent signals, 18 skeletal carbon resonances appeared in the ^{13}C NMR spectrum of **2**, which, in combination with its ^{1}H NMR data, suggested that **2** is a dihydrobenzo[*b*]furan neolignan.[23,24] In the HMBC spectrum of **2**, H_2-9 (δ_H 4.45 and 4.09) and the methyl signal (δ_H 2.04, 3H, s, OAc) correlated to the carbonyl carbon (δ_C 170.8, s, OAc) of the acetoxyl group, and the methoxy signal at δ_H 3.40 (3H, s, OMe-9′) correlated to C-9′ at δ_C 73.2. These observations indicated that the acetoxyl group was attached to C-9 and a methoxy group was located at C-9′. The deuterium exchangeable singlet at δ_H 5.63 (1H, s, OH-3′) was not correlated with any carbon signals in the HMQC spectrum of **2**, while this proton singlet was correlated to C-2′, C-3′, and C-4′ in the HMBC spectrum of **2**. This permitted the assignment of the hydroxy group at C-3′. The *trans* relationship between H-7 and H-8 was determined by comparison of the coupling constant and the chemical shifts of these two protons with those of reported analogues.[23] Accordingly, compound **2** was assigned as (−)-*trans*-9-acetyl-4,9′-di-*O*-methyl-3′-de-*O*-methyldehydrodiconiferyl alcohol.

Five known compounds, 7,7′-dihydroxy-6,8′-bicoumarin (bicoumol) (**3**),[25] 3,4-dimethoxycinnamaldehyde (**4**),[26] 6-hydroxy-7-methoxycoumarin (isoscopoletin),[27] *N*-butylaniline,[28] and vanillin,[29] were isolated along with compounds **1** and **2**. The structures of these known compounds were identified by physical and spectroscopic data measurement (^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) and by comparing the data obtained with those of published values. Full ^{1}H and ^{13}C NMR data assignments of bicoumol (**3**) are reported in the Experimental Section for the first time.

The potential of all isolates to induce quinone reductase (QR) in murine Hepa1c1c7 hepatoma cells[5] and to inhibit the transformation of murine epidermal JB6 cells[6] was evaluated according to previous protocols. The results indicated that only 3,4-dimethoxycinnamaldehyde (**4**) was significantly active in these assays with a CD (concentration to double induction) value of 9.5 μg/mL (52.8 μM) (QR assay) and an IC_{50} value of 2.3 μg/mL (12.8 μM) (JB6 assay), respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectral data were recorded at room temperature on Bruker Avance DPX-300 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. EIMS and HREIMS data were obtained on a Finnigan/MAT 90/95 sector-field mass spectrometer, and HRFABMS were obtained on a VG 7070E-HF sector-field mass spectrometer. X-ray crystallographic analysis data collection for compound **1** was carried out on an Enraf-Nonius Kappa CCD area detector with a rotating anode Mo X-ray tube. The direct method SIR-92 was used to locate non-hydrogen atoms,[30] and the WinGX package[31] was used for completing the structure determination, while ORTEP[32] was used to generate Figure 1. Column chromatography was carried out with Si gel G (Merck, 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thickness Merck Si gel 60 F_{254} aluminum plates, while preparative thin-layer chromatography was performed on precoated 1000 μm thickness Merck Si gel 60 F_{254} glass plates.

Plant Material. The stem wood of *Euphorbia quinquecostata* was collected in Tanzania by Z.H.M. in February 1999. A voucher specimen (PA0177) has been deposited at the University of Illinois Pharmacognosy Field Station, Downers Grove, IL.

Extraction and Isolation. The dried and milled stem wood (9.5 kg) was extracted by maceration with MeOH three times (3 × 20 L) at room temperature, one day each time. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract (475 g) was suspended in H_2O (1000 mL), then partitioned in turn with petroleum ether (2 × 1000 mL) and EtOAc (3 × 1000 mL), to afford dried petroleum ether- (131.5 g), EtOAc- (29.7 g), and H_2O-soluble residues.

The EtOAc-soluble extract was subjected to Si gel column chromatography by elution with petroleum ether−EtOAc (from 20:1 to 1:1, then pure EtOAc) to give six fractions (F01−F06). Fraction F02 (640 mg) was chromatographed over a Si gel column (2.5 × 45 cm) and eluted with $CHCl_3$−acetone (20:1 to 4:1) to give three subfractions (F0201−F0203). Subfractions F0201 and F0202 were separately purified by the same isocratic solvent system (hexanes−EtOAc, 6:1) over Si gel columns (1.2 × 20 cm) and yielded vanillin (6 mg) and 3,4-dimethoxycinnamaldehyde (**4**, 7.5 mg), respectively. Fractions F03 (2.4 g) and F04 (2.7 g) were combined and separated over a further Si gel column (3.8 × 60 cm), using a gradient solvent system of petroleum ether−acetone (from 6:1 to 1:1), to give four subfractions (F0301−F0304). Subfraction F0301 (328 mg) was then further purified over a Si gel column (1.2 × 20 cm), using hexanes−EtOAc (5:1) as solvent, to afford 6-hydroxy-7-methoxycoumarin (isoscopoletin, 152 mg). Subfraction F0302 (1.13 g) was then subjected to Si gel column (2.5 × 45 cm) chromatography and eluted with a gradient $CHCl_3$−MeOH solvent system (from 100:1 to 5:1), to give, in order of polarity, an additional quantity of isoscopoletin (87 mg), a further subfraction F030202, and a second subfraction (F030203) containing semipure compound **1**. Subfraction F030202 was finally purified by preparative TLC (Merck 60 Å Si gel, 20 × 20 cm, 1000 μm) and developed with $CHCl_3$−MeOH (30:1), yielding compound **2** (3 mg, $R_f = 0.60$). Pure compound **1** (123 mg) was obtained as colorless needles from a $CHCl_3$−MeOH (∼1:1) solution of F030203. Subfraction F0303 (127 mg) was separated over a Sephadex-LH 20 column (2.5 × 45 cm) and eluted with pure MeOH, to yield *N*-butylaniline (10 mg). Bicoumol (**3**, 7,7′-dihydroxy-6,8′-bicoumarin, 58 mg) was obtained as a white amorphous powder from a $CHCl_3$−MeOH (∼2:1) solution of subfraction F0304.

3α,12α-Dihydroxy-*ent*-8(14),15-isopimaradien-18-al (1): colorless needles ($CHCl_3$−MeOH, 1:1), mp 149−150 °C, $[\alpha]_D^{23}$ +7.0° (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (3.40), 242 (2.82) nm; IR (film) ν_{max} 3410 (OH), 1717 (−CHO), 1045 (double bond), 1000, 918, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.41 (1H, s, H-18), 5.76 (1H, dd, J = 17.5, 10.7 Hz, H-15), 5.09−5.17 (3H, overlapped, H-16*cis*, H-16*trans*, and H-14), 3.81 (1H, dd, J = 11.6, 4.1 Hz, H-3), 3.57 (1H, dd, J = 12.2, 4.1 Hz, H-12), 2.25 (1H, m, H-7a), 2.00−2.05 (2H, m, H-7b and H-9), 1.73−1.83 (3H, m, H-1a, H-2a, and H-11a), 1.60−1.70 (1H, m, H-2b), 1.47−1.59 (2H, m, H-5, H-11b), 1.28 (1H, ddd, J = Hz, H-1b), 1.11 (3H, s, CH_3-19), 1.05−1.11 (2H, m, H_2-6, partly overlapped with the signals of CH_3-17 and CH_3-19), 1.06 (3H, s, CH_3-17), and 0.87 (3H, s, CH_3-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.7 (d, C-18), 145.9 (d, C-15), 135.7 (s, C-8), 128.8 (d, C-14), 113.9 (t, C-16), 73.1 (d, C-12), 72.2 (d, C-3), 55.1 (s, C-4), 51.2 (d, C-9), 46.4 (d, C-5), 43.1 (s, C-13), 36.8 (s, C-10), 36.6 (t, C-1), 34.1 (t, C-7), 26.5 (t, C-2), 26.1 (t, C-11), 23.7 (t, C-6), 17.5 (q, C-17), 15.0 (q, C-20), 9.3 (q, C-19); EIMS *m/z* 318 $[M]^+$ (11), 300 $[M - H_2O]^+$ (21), 289 $[M - CHO]^+$ (42), 271 (27), 229 (25), 227 (24), 199 (25), 187 (28), 185 (33), 173 (28), 159 (53), 145 (49), 133 (100), 129 (92), 105 (95), 91 (83), 81 (57), 67 (42); HREIMS *m/z* 318.2183 (calcd for $C_{20}H_{30}O_3$, 318.2195).

X-ray Crystallography of 1. A colorless crystal was obtained from $CHCl_3$−MeOH (∼1:1). Crystal size: 0.22 × 0.30 × 0.38 mm. Cell parameters: a = 6.1536(3) Å; b = 14.7224(6) Å, β = 93.8425(16)°; c = 10.6662(6) Å; V = 964.14(8) $Å^3$, space group $P2_1$, Z = 2, D_{calc} = 1.207 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.081 mm^{-1}, $F(000)$ = 384, T = 150(1) K. Data collection yielded 5712 reflections resulting in 3080 unique, averaged reflections, 2841 with $I > 2\sigma_I$. Full-matrix least-squares refinement led to a final R and R(all) values of 0.0416 and 0.0467, and GOF = 1.077. 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 181225. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1 by a Convenient Mosher Ester Procedure. Compound **1** (2.0 mg) was transferred into a clean NMR tube and was dried completely under the vacuum of an oil pump. Deuterated pyridine (0.5 mL) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) were added into the NMR tube immediately under a N_2 gas stream, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand at room temperature and monitored every 2 h by ^1H NMR. The reaction was found to be complete after 6 h. ^1H NMR data of the (*R*)-MTPA ester derivative (**1r**) of **1** (360 MHz, pyridine-d_5; data were obtained from the reaction NMR tube directly and were assigned on the basis of the correlations of the $^1H-^1H$ COSY spectrum): δ 9.655 (1H, s, H-18), 5.817 (1H, dd, J = 17.4, 10.7 Hz, H-15), 5.571 (1H, dd, J = 11.8, 4.3 Hz, H-3), 5.366 (1H, dd, J = 12.4, 4.0 Hz, H-12), 5.202, (1H, br s, H-14), 5.090 (1H, d, J = 10.7 Hz, H-16*cis*), 5.057 (1H, d, J = 17.4 Hz, H-16*trans*), 1.185 (3H, s, CH_3-17), 1.156 (3H, s, CH_3-19), 0.752 (3H, s, CH_3-20). In the manner described for **1r**, another portion of compound **1** (2.0 mg) was reacted in a second NMR tube with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) at room temperature for 6 h using deuterated pyridine (0.5 mL) as solvent, to afford the (*S*)-MTPA derivative of **1** (**1s**). ^1H NMR data of **1s** (360 MHz, pyridine-d_5): δ 9.602 (1H, s, H-18), 5.913 (1H, dd, J = 17.4, 10.8 Hz, H-15), 5.568 (1H, dd, J = 11.9, 4.5 Hz, H-3), 5.378 (1H, dd, J = 12.5, 4.0 Hz, H-12), 5.188 (1H, br s, H-14), 5.203 (1H, d, J = 17.4 Hz, H-16*trans*), 5.179 (1H, d, J = 10.8 Hz, H-16*cis*), 1.222 (3H, s, CH_3-17), 1.109 (3H, s, CH_3-19), 0.714 (3H, s, CH_3-20).

Purification of the (*R*)- and (*S*)-MTPA Ester Derivatives (1r and 1s) of 1. To confirm the absolute configuration of **1** determined by the above procedure, the reaction mixtures were transferred from the NMR tubes and purified over a very small column (0.5 g Si gel) separately, using $CHCl_3$−MeOH (100:1) as elution solvent, affording pure **1r** and **1s**, respectively. ^1H NMR of **1r** (500 MHz, $CDCl_3$, data were assigned on the basis of the correlations of its $^1H-^1H$ COSY and NOESY spectra): δ 9.380 (1H, s, H-18), 5.633 (1H, dd, J = 17.5, 10.6 Hz, H-15), 5.524 (1H, dd, J = 11.8, 4.5 Hz, H-3), 5.178 (1H, br s, H-14), 5.075 (1H, dd, J = 12.4, 4.2 Hz, H-12), 4.970 (1H, d, J = 10.7 Hz, H-16*cis*), 4.920 (1H, d, J = 17.5 Hz, H-16*trans*), 2.158 (1H, br t, J = 8.6 Hz, H-9), 1.900 (1H, m, H-11a), 1.876 (1H, m, H-2a), 1.744 (1H, m, H-11b), 1.702 (1H, m, H-2b), 1.656 (1H, dd, J = 12.7, 2.3 Hz, H-5), 1.100 (3H, s, CH_3-19), 1.048 (3H, s, CH_3-17), 0.886 (3H, s, CH_3-20). ^1H NMR of **1s** (500 MHz, $CDCl_3$, data were assigned on the basis of the correlations of its $^1H-^1H$ COSY and NOESY spectra): δ 9.331 (1H, s, H-18), 5.730 (1H, dd, J = 17.5, 10.7 Hz, H-15), 5.567 (1H, dd, J = 11.9, 4.4 Hz, H-3), 5.164 (1H, br s, H-14), 5.088 (1H, d, J = 10.7 Hz, H-16*cis*), 5.062 (overlapped with H_2-16, H-12), 5.034 (1H, d, J = 17.5 Hz, H-16*trans*), 2.156 (1H, br t, J = 8.6 Hz, H-9), 1.956 (1H, m, H-2a), 1.860 (1H, m, H-11a), 1.827 (1H, m, H-2b), 1.643 (1H, dd, J = 12.6, 2.4 Hz, H-5), 1.594 (1H, m, H-11b), 1.054 (3H, s, CH_3-19), 1.093 (3H, s, CH_3-17), 0.878 (3H, s, CH_3-20).

(−)-*trans*-9-Acetyl-4,9′-di-*O*-methyl-3′-de-*O*-methylde-hydrodiconiferyl alcohol (2): yellowish oil, $[\alpha]_D^{23}$ −73.3° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (3.68), 279 (3.38), 339 (3.12) nm; IR (film) ν_{max} 3452 (OH), 1737 (OAc), 1603, 1512, 1459, 1235 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.88−6.91 (5H, m, Ar−H), 6.56 (1H, d, J = 15.8 Hz, H-7′), 6.16 (1H,

dt, J = 15.8, 6.0 Hz, H-8′), 5.63 (1H, s, OH-3′), 5.48 (1H, d, J = 7.5 Hz, H-7), 4.45 (1H, dd, J = 11.1, 5.6 Hz, H-9a), 4.32 (1H, dd, J = 11.1, 8.7 Hz, H-9b), 4.09 (2H, br d, J = 6.0 Hz, H_2-9′), 3.91 (3H, s, Ar−OMe), 3.88 (3H, s, Ar−OMe), 3.79 (1H, m, H-8), 3.40 (3H, s, OMe-9′), 2.04 (OAc); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8 (s, OAc), 146.7, 146.2, 144.4 (s, C-3, C-4, and C-4′),145.8 (s, C-3′), 132.5 (d, C-7′), 132.3 (s, C-1), 131.0 (s, C-1′), 127.6 (s, C-5′), 124.0 (d, C-8′), 119.6 (d, C-6), 115.0 (d, C-6′ or C-5), 114.3 (d, C-2′), 110.4 (d, C-6′ or C-5), 108.6 (d, C-2), 88.8 (d, C-7), 73.2 (t, C-9′), 65.4 (t, C-9), 58.0 (d, C-8), 56.0 (q, Ar−OMe × 2), 50.3 (q, OMe-9′), 20.8 (q, OAc); EIMS m/z 414 $[M]^+$ (62), 384 $[M - OMe]^+$ (5), 354 $[M - AcOH]^+$-(100), 339 (25), 291 (20), 181 (23), 151 (29), 137 (24), 71 (24); HREIMS m/z 414.1689 (calcd for $C_{23}H_{26}O_7$, 414.1679).

Bicoumol (7,7′-dihydroxy-6,8′-bicoumarin) (3): white amorphous powder, mp 284−287 °C [lit.[25] 293−294 °C]; UV (MeOH) λ_{max} (log ϵ) 211 (4.08), 258 (3.54), 327 (4.01) nm; IR (film) ν_{max} 3276 (OH), 1700 (carbonyl carbon), 1594, 1387, 1152, 1129 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.99 (1H, d J = 9.6 Hz, H-4′), 7.96 (1H, d, J = 9.6 Hz, H-4), 7.56 (1H, d, J = 8.3 Hz, H-5′), 7.48 (1H, br s, H-5), 6.96 (1H, d, J = 8.3 Hz, H-6′), 6.88 (1H, s, H-8), 6.25 (1H, d, J = 9.6 Hz, H-3), 6.20 (1H, d, J = 9.6 Hz, H-3′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 161.0 (s, C-2′), 160.5 (s, C-2), 159.3, 159.0 (s, C-7 and C-7′), 155.6 (s, C-8a), 153.3 (s, C-8′a), 144.9 (d, C-4′), 144.5 (d, C-4), 131.7 (d, C-5), 128.7 (d, C-5′), 117.2 (s, C-6), 112.7 (d, C-6′), 111.8 (s, C-8′), 111.6 (d, C-3), 111.4, 111.3 (s, C-4a and C-4′a), 111.1 (d, C-3′), 102.1 (d, C-8); EIMS m/z 322 $[M]^+$ (72), 304 (12), 294 (10), 119 (20), 105 (25), 69 (100), 55 (53); positive HRFABMS m/z 323.0564 $[M + H]^+$ (calcd for $C_{18}H_{11}O_6$, 323.0556).

Evaluation of Quinone Reductase-Inducing Ability of Isolates. This method was described previously.[5] Compounds **1**−**4** were tested at various concentrations ranging up to 20 μg/mL.

Evaluation of Transformation of Murine Epidermal JB6 Cells. This method was described previously.[6] Compounds **1**−**4** were tested at various concentrations ranging up to 4 μg/mL.

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Supporting Information Available: The selected HMBC and NOESY correlations for compound **1** and HMBC correlations for compound **2**, ^{1}H NMR spectra of the (*R*)- and (*S*)-MTPA esters of compound **1** in both pyridine-d_5 (obtained by running reaction NMR tubes directly) and $CDCl_3$ (after purification), and the ^{1}H and ^{1}H−^{1}H COSY NMR spectra of philadelphicalactone A in pyridine-d_5 (obtained by running in reaction NMR tubes directly). This material is available free of charge via the Internet at http://pubs.acs.org.

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