Published in final edited form as:

J Nat Prod. 2019 October 25; 82(10): 2852–2858. doi:10.1021/acs.jnatprod.9b00596.

Prenylated Acetophloroglucinol Dimers from *Acronychia trifoliolata*: Structure Elucidation and Total Synthesis

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Abstract

The isolation of 12 secondary metabolites, including seven new acetophenone monomers, from the 50% CH₃OH/CH₂Cl₂ extract (N089419-L/6) of *Acronychia trifoliolata* was reported previously. In the present work, three new prenylated acetophenone dimers (1–3) and five known dimers (4–8) were isolated, and their structures were elucidated by using various NMR spectroscopic techniques and HRMS. Among the new dimers, an unprecedented 4-isobutyl-3-isopropyltetrahydro-2*H*-pyran ring was observed in the structure of 1. This study is the first to report the formation of a 2*H*-pyran ring between two prenylated acetophloroglucinols. Only four related dimers have been reported before, and they were formylated phloroglucinol dimers from the family Eucalypteae. Compounds 2 and 3 are acrovstone-like dimers, and the structure of 3 was confirmed by total synthesis. The evaluation of the antiproliferative activity of isolated and synthesized acrovstone-like dimers indicated that a double bond in the prenyl-like moiety as

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The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnat-prod.9b00596. NMR spectra and HFFABMS data for 1–3, NCI60 panel screening data of plant extract, and ¹H NMR of synthesized 3 (PDF)

found in the more active compounds might be important for mediating activity, while the pendant isobutyl group seems to be less important.

Graphical Abstract

The genus *Acronychia* belongs to the family Rutaceae in the major group Angiosperms (flowering plants). Among 49 species,¹ only half of these have been investigated phytochemically and pharmacologically, even though many species are used in traditional folk medicine for the treatment of diarrhea, cough, asthma, sores, ulcers, itchy skin, pain, and rheumatism.^{2,3} Thus, plants in this genus merit further phytochemical research for the discovery of potential new bioactive natural products.

Prenylated acetophenones^{3–11} are characteristic secondary metabolites of the family Rutaceae. Especially, the genus *Acronychia* is known to biosynthesize dimeric compounds, in which two prenylated acetophenones are connected by a methylene with a pendant isobutyl group. Seven dimers, acrovestone,¹² acrovestenol,¹³ acrofolione A,⁷ acrofolione B,⁷ acropyrone,¹⁴ acropyranol A,¹⁴ and acropyranol B,¹⁴ have been isolated, and all of them exhibit some cytotoxicity against cancer cells.

Our previous phytochemical research led to the identification of new noncytotoxic prenylated acetophenone monomers from *A. trifoliolata* Zoll. & Moritzi,¹¹ a rainforest plant distributed from Java and Christmas Island to the Solomon Islands. In the present work, a 50% CH₃OH/CH₂Cl₂ extract of *A. trifoliolata* (N089419-L/6), obtained from the National Cancer Institute (NCI), displayed significant antiproliferative activity against NCI60 panels of human tumor cell lines (Figure S36). Subsequent isolation work led to the characterization of three new prenylated acetophloroglucinol dimers, 1–3.

RESULTS AND DISCUSSION

A 50% CH₃OH/CH₂Cl₂ extract of the bark of *A. trifoliolata* (4.9 g) was fractionated with ethyl acetate and water to provide ethyl acetate- and water-soluble fractions. The ethyl acetate-soluble fraction was separated by a combination of medium-pressure liquid chromatography (MPLC), silica gel column chromatography, and preparative TLC techniques to give three new prenylated acetophloroglucinol dimers, acrotrifoliolane A (1), acrofolione C (2), and acrofolional (3), together with five known compounds, acrovestone (4),³ acropyrone (5),¹³ acropyranols A (6)⁷ and B (7),¹⁴ and acrovestenol (8), of which the latter three were isolated as racemates (Figure 1).

The ^1H NMR spectrum of acrotrifoliolane A (1) showed signals assignable to two olefins (δ_{H} 5.24, 5.05), a deshielded oxymethine (δ_{H} 5.14), two methoxy group methyls (δ_{H} 3.77, 3.75), two deshielded methylenes (δ_{H} 3.34, 3.22), a deshielded methine (δ_{H} 3.29), two acetyl methyls (δ_{H} 2.73, 2.71), three methines (δ_{H} 2.26, 1.71, 1.48), a methylene (δ_{H} 1.33, 1.43), four tertiary methyls (δ_{H} 0.96, 0.91, 0.87, 0.79), and three hydrogen-bonded hydroxy protons (δ_{H} 13.68, 13.29, 7.96) (Table 1). The ^{13}C NMR spectrum of 1 (Table 1) indicated 38 carbons including two ketone carbonyl carbons (δ_{C} 204.4, 203.9), two pairs of olefinic carbons (δ_{C} 132.0, 131.9, 122.5, 123.0), 12 aromatic carbons (δ_{C} 161.6, 161.4, 161.3, 161.1, 160.4, 158.7, 117.9, 116.3, 115.4, 110.2, 111.0, 108.7), and an oxymethine (δ_{C} 79.1), which suggested the presence of two phenyl rings. These signals were very similar to those of sideroxylonal C, isolated from *Eucalyptus albens*¹⁵ and *E. melliodora*,¹⁶ except for the two pairs of prenyl groups and two acetyl groups present instead of four formyl groups as well as the two methoxy groups replacing two hydroxy groups. Furthermore, compound 1 showed a protonated molecular ion peak at m/z 637.3744 in the HRFABMS, corresponding to the molecular formula $\text{C}_{38}\text{H}_{53}\text{O}_8$, which suggested that this compound is a dimeric derivative of 1-[2,4-dihydroxy-6-methoxy-3,5-bis(3-methyl-2-buten-1-yl)phenyl]ethanone, previously isolated from the same extract.¹¹ The planar structure of 1 was confirmed by 2D NMR experiments, including the ^1H – ^1H COSY, HMQC, HMBC, and NOESY spectra (Figures 2 and 3). Data from the ^1H – ^1H COSY experiment confirmed the presence of two prenyl groups and an aliphatic 10-carbon unit. The connections of the two phenyl rings with the 10-carbon unit were deduced from HMBC correlations. The NOESY correlations of the methoxy group methyl protons (δ_{H} 3.75) with the methylene protons of a prenyl moiety (δ_{H} 3.22) and the acetyl methyl protons (δ_{H} 2.73) indicated the latter two groups and the methoxy group to be close in space. Similarly, the NOESY correlations of the other methoxy methyl protons (δ_{H} 3.77) with the methylene protons of a prenyl moiety (δ_{H} 3.34) and the acetyl methyl protons (δ_{H} 2.71) as well as of the acetyl methyl protons (δ_{H} 2.71) with a phenolic proton (δ_{H} 13.68) determined the arrangement of the substituents on the phenyl ring. The relative configuration of 1 was determined from the NOESY data (Figure 3). The correlations between H-1''', H-5''', and the OH at C-2''' indicated that the phenyl ring at C-1''' and an isopropyl group at C-2''' face in opposite directions (i.e., are *trans* oriented). In addition, the isopropyl and isobutyl groups were also *trans* oriented based on NOESY correlations between H-2', H-2''', and the OH at C-4'''. The assigned relative configuration was supported by a comparison of the H-1', H-1''', and H-2''' coupling constants with those of the related phloroglucinol dimers, sideroxylonals A, B, and C.^{15–17}

It should be noted that sideroxylonals A, B, and C^{15–17} as well as loxophlebal A,¹⁸ four related phloroglucinol dimers with a 4-isobutyl-3-isopropyltetrahydro-2*H*-pyran unit, were isolated only from the genus *Eucalyptus*^{15–21} in the family Myrtaceae. They were all racemic with the same planar structure derived from formylated phloroglucinols. Therefore, compound 1 is the first prenylated acetophloroglucinol dimer identified from a plant other than in the Myrtaceae. As proposed before,^{17,18} the 2*H*-pyran ring could be formed biosynthetically by a Diels–Alder cyclization between the related α -quinone methide as a diene with the styrene monomer as a dienophile (Figure 4).

The ^1H NMR spectrum (Table 1) of 2 was closely related to that of known acrofolione B, previously isolated as a racemate from *A. trifoliolata*.⁷ Some signals were broadened in the

¹H and ¹³C NMR spectra because of freely rotatable aryl moieties in 2, making the structural elucidation difficult. Although the connectivities could not be determined from the spectra in (CD₃)₂SO, confirmation was provided by measuring the spectra in several deuterated solvents, such as C₆D₆ and (CD₃)₂CO (Figure 2). The resultant planar structure established was identical to that of acrofolione B. Acrofolione B was originally elucidated by using ¹H NMR, DQFCOSY, and NOE difference spectra, and the assigned fusion position of the dihydrofuran ring was not conclusive. In another study,¹⁴ this position was determined by comparison of the NOE correlations between acrofoliones A and B with reference to acropyranols A and B. Although 2D NMR analysis indicated that 2 and acrofolione B have the same planar structure, the chemical shift patterns of the two compounds were different (Table S1, Supporting Information). Thus, compound 2 (acrofolione C) was proposed as a diastereomer of acrofolione B.

Compound 3 was isolated in a limited quantity, insufficient to obtain a clear ¹³C NMR spectrum. However, the ¹H NMR spectrum of 3 resembled that of 4 except for the absence of signals for a prenyl group and presence of a signal for an aldehyde proton at 10.16 ppm (Figure S32, Supporting Information). Based on the correlations in ¹H–¹H COSY and NOESY data, compound 3 (acrofolional) was proposed as a carbaldehyde derivative of acrovestone-type acetophenone dimers. Its structure was confirmed by total synthesis.

To synthesize 3, acetophloroglucinol (9) was converted initially to the prenylated acetophenone 10.²² The preparation of acetophenone homodimers was previously reported^{23,24} using a Friedel–Craft reaction of electron-rich aryl groups with alkyl aldehydes (Scheme 1). Although heterodimer syntheses have not been reported, we performed the Friedel–Craft reaction of acetophenone 10 with isovaleraldehyde in the presence of AlCl₃ under heating at 120 °C. The reaction time was controlled carefully to avoid the production of homodimer 13. After the disappearance of starting material by TLC, the aryl derivative 9 was added to the reaction mixture. The desired heterodimer 12 was obtained in 19% yield together with homodimer 13 (22%) and 10 (11%). The treatment of 12 under Vilsmeier–Haack conditions produced 3 in 28% yield. The ¹H NMR (Figure S38, Supporting Information), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) data were identical to those of the isolated compound.

To determine the structure–activity relationship of the pendant isobutyl group, the related derivatives 15 and 16 were also synthesized through dimethylamine 14 produced by Eschenmoser salt and 10 (Scheme 2).

The isolated acetophenone dimers 2–8 as well as synthetic derivatives 12, 13, 15, and 16 were evaluated for antiproliferative activity against four tumor cell lines (Table 2). Among them, compounds 4, 5, 12, 15, and 16 inhibited the tumor cell growth with IC₅₀ values of around 5 μM against all tested cell lines, whereas 8 and 13 showed lower activities. Since compounds 2, 6, and 7 were less potent, a double bond in the prenyl-like moiety as found in the more active compounds might be important for the antiproliferative activity. The comparison between 12 and 15 as well as 13 and 16 indicated that a pendant isobutyl moiety is not crucial for this type of biological activity.

In this study, eight prenylated acetophenone dimers were isolated, including three new compounds, acrotrifoliolane A (1), acrofolione C (2), and acrofolional (3), from a rainforest plant, *A. trifoliolata*. Their chemical structures were characterized by spectroscopic and spectrometric data. All compounds were isolated as racemates similarly to acetophenone monomers and dimers purified from other *Acronychia* species. The total synthesis of 3 was accomplished to confirm its structure. Finally, the antiproliferative activities of isolated compounds were also evaluated.

EXPERIMENTAL SECTION

General Experimental Procedures.

Optical rotations were recorded on a JASCO P-2200 digital polarimeter. Infrared spectra (IR) were measured with a Shimadzu FTIR-8700 instrument for samples in CHCl₃. NMR spectra were taken on a JEOL JMN-ECA600 spectrometer and JMN-ECS400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are expressed as δ values. High-resolution mass spectrometric data were obtained on a JEOL JMS-700 (FAB) or a JMS-T100TD (DART) mass spectrometer. Analytical and preparative TLC was carried out on precoated silica gel 60F₂₅₄ and RP-18F₂₅₄ plates (0.25 or 0.50 mm thickness; Merck). MPLC was performed with silica gel and C₁₈ cartridges (Biotage, Uppsala, Sweden).

Plant Material.

The bark of *Acronychia trifoliolata* was collected in Bali, Indonesia, at an altitude of 1200 m on July 5, 1994, by Dr. Djaja D. Soejarto and identified by the taxonomist, A. McDonald. A voucher specimen (#U44Z-4773) is housed at the Smithsonian Institution, Washington, DC. A voucher extract (N089419-L/6) was deposited at the NCI and at Kanazawa University.

Extraction and Isolation.

A 50% CH₃OH/CH₂Cl₂ extract of the bark of *A. trifoliolata* (4.9 g), provided by NCI, was dissolved into water and partitioned with EtOAc to yield EtOAc-soluble (3.8 g) and H₂O-soluble portions. The EtOAc-soluble fraction (3.7 g) was dissolved in EtOAc to remove precipitated material and then was subjected to silica gel MPLC (KP-Sil 100 g), with a CH₃OH/CHCl₃ gradient system [CHCl₃ (5 fractions) → 2% CH₃OH/CHCl₃ (5 fractions) → 10% CH₃OH (5 fractions) → CH₃OH (1 fraction); collection per 100 mL], to yield 16 fractions.

Fraction 2 (750 mg) was subjected to silica gel MPLC (SNAP HP-Sil 25 g), with the eluents EtOAc/*n*-hexane [1:19, 12 column volumes (CVs) and 1:4, 5 CVs], followed by CH₃OH, to yield 18 subfractions. Subfraction 4 (50.6 mg) was purified by reversed-phase MPLC (KP-C18-HS 12 g) with 60% acetone/H₂O, reversed-phase preparative TLC with CH₃OH and 90% acetone/H₂O, and preparative TLC with toluene to yield 1 (0.5 mg) and acropyrone (6, 1.6 mg). Subfractions 6–8 were combined (114 mg), then were purified by Si gel MPLC (SNAP Ultra 10 g) with toluene and preparative TLC with 10% EtOAc/toluene, to yield 2 (9.8 mg). Subfractions 10–12 were combined (57.1 mg), then were purified by preparative TLC with 10% EtOAc/toluene to yield acrovestone (4, 11.5 mg). Subfractions 13–15 were combined (97.8 mg) and then purified by reversed-phase MPLC (KP-C18-HS

12 g), with an acetone/H₂O stepwise gradient system [50%, 70%, 90%, and 100%; 6 CVs each], reversed-phase preparative TLC with 80% acetone/H₂O, and preparative TLC with 5% acetone/toluene, to yield acrovestenol (5, 2.9 mg). Subfractions 16 and 17 were combined (72.0 mg) and were purified by Si gel MPLC (SNAP Ultra 10 g) with toluene followed by 10% EtOAc/toluene, reversed-phase preparative TLC with 80% acetone/H₂O, and preparative TLC with 20% acetone/*n*-hexane, to yield acropyranol A (7, 0.5 mg) and acropyranol B (8, 1.1 mg). Subfraction 18 (251 mg) was purified by reversed-phase MPLC (KP-C18-HS 12 g) with a CH₃OH/H₂O stepwise gradient system [70%, 80%, 90%, 100%; 6 CVs each], preparative TLC with 50% EtOAc/*n*-hexane, reversed-phase preparative TLC with acetone/H₂O/CH₃COOH (80:19:1), and preparative TLC with 25% EtOAc/toluene, to yield 3 (0.5 mg).

Acrotrifoliolane A (1): yellow, amorphous solid; $[\alpha]_D^{22} +0.24$ (*c* 0.03, CH₃OH); IR $\nu_{\max}$ (CHCl₃) 3396, 2956, 2927, 1739, 1613, 1455, 1416, 1366, 1268; 1192, 1109, 1038, 978 cm⁻¹; ¹H and ¹³C NMR, Table 1; FABMS *m/z* 637, HRFABMS *m/z* 637.3744 [M + H]⁺ (calcd for C₃₈H₅₃O₈, 637.3740).

Acrofolione C (2): yellow, amorphous solid; $[\alpha]_D^{21} -2.77$ (*c* 0.06, CH₃OH); IR $\nu_{\max}$ (CHCl₃) 3260, 2956, 2929, 2868, 1615, 1454, 1366, 1321; 1270, 1190, 1097, 975 cm⁻¹; ¹H and ¹³C NMR, Table 1; FABMS *m/z* 571, 319; HRFABMS *m/z* 571.2900 [M + H]⁺ (calcd for C₃₂H₄₃O₉, 571.2907).

Acrofolional (3): yellow, amorphous solid; $[\alpha]_D^{17} +0.48$ (*c* 0.03, CHCl₃); IR $\nu_{\max}$ (CHCl₃) 3366, 2956, 2925, 1614, 1469, 1367; 1320, 1214, 1100, 974 cm⁻¹; ¹H NMR, Table 1; FABMS *m/z* 515, 319; HRFABMS *m/z* 515.2247 [M + H]⁺ (calcd for C₂₈H₃₅O₉, 515.2281).

1-{3-[1-(3-Acetyl-2,4,6-trihydroxyphenyl)-3-methylbutyl]-2,4-dihydroxy-6-methoxy-5-(3-methylbut-2-en-1-yl)phenyl}ethanone (12).—To a solution

of 10 (32.4 mg, 0.13 mmol) in anhydrous toluene (1.0 mL) was added anhydrous AlCl₃ (2.7 mg, 0.02 mmol) at room temperature. After stirring for 15 min, isovaleraldehyde (14 μ L, 0.13 mmol) was added and refluxed for 1 h. After consumption of 10 (TLC), the mixture was cooled to room temperature and 9 (22.1 mg) was added. After refluxing for 9 h, the stirring was continued for 16 h at 80 °C. The mixture was extracted with CH₂Cl₂, and the organic layer was washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:5–1:2) to afford the target compounds 12 (11.7 mg, 19%) and 13 (8.8 mg, 22%) as pale yellow oils along with recovered starting material 10 (3.5 mg, 11%): $[\alpha]_D^{21} +1.74$ (*c* 0.30, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 15.7 (1H, brs), 15.6 (1H, brs), 9.95 (1H, brs), 9.15 (1H, brs), 5.88 (1H, brs), 5.21–5.18 (1H, m), 4.71 (1H, t, *J* = 7.8 Hz), 3.71 (3H, s), 3.30 (2H, brs), 2.71 (3H, s), 2.68 (3H, s), 2.24 (1H, brs), 2.08 (1H, brs), 1.77 (3H, s), 1.70 (3H, s), 1.41 (1H, 7, *J* = 6.0 Hz), 0.88–0.87 (6H, m); ¹³C NMR (100 MHz, CDCl₃) δ 204.2, 160.2, 122.9, 62.7, 32.5, 30.7, 28.2, 27.0, 25.7, 23.1, 22.7, 22.4, 18.0; HRFABMS *m/z* 486.2210 [M]⁺ (calcd for C₂₇H₃₄O₈, 486.2254).

Synthesis of Acrofolional (3).

To a solution of dimethylformamide (DMF, 0.1 mL) in anhydrous CH_2Cl_2 (0.18 mL) was added POCl_3 (0.12 mL) at 0 °C under Ar, and the mixture was stirred for 20 min to generate the Vilsmeier reagent. A solution of 12 (6.5 mg, 0.01 mmol) in anhydrous CH_2Cl_2 (0.6 mL) was added to the Vilsmeier reagent (0.05 mL) at 0 °C under Ar and stirred for 1 h. The mixture was quenched with water (1.0 mL), and stirring was continued for 30 min at room temperature. The mixture was extracted three times with EtOAc, and the combined organic layers were washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The crude product was purified using preparative TLC with EtOAc to afford 3 (1.9 mg, 28%) as a yellow, amorphous solid: $[\alpha]_D^{21} -0.48$ (c 0.08, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 17.1 (1H, brs), 16.1 (1H, brs), 14.5 (1H, brs), 10.2 (1H, brs), 5.20–5.17 (1H, m), 4.70 (1H, t, J = 10.4 Hz), 3.73 (3H, s), 3.30 (2H, brs), 2.74 (3H, s), 2.73 (3H, s), 1.77 (3H, s), 1.70 (3H, s), 1.44–1.36 (1H, m), 0.88 (6H, brs); ^{13}C NMR (150 MHz, CDCl_3) δ 193.5, 62.7, 32.3, 29.7, 26.9, 25.7, 22.5, 18.0; HRFABMS m/z 515.2234 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{28}\text{H}_{35}\text{O}_9$, 515.2281).

1-{3-[(Dimethylamino)methyl]-2,4-dihydroxy-6-methoxy-5-(3-methylbut-2-en-1-yl)phenyl}ethanone (14).—To a solution of 10 (281.9 mg, 1.13 mmol) in CHCl_3 (20 mL) was added Eschenmoser's salt (289.9 mg, 1.57 mmol), and the mixture was refluxed for 30 min under N_2 . The mixture was cooled to room temperature, quenched with water (20 mL), and then extracted with CHCl_3 . The combined organic layers were washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2–10:0) to afford 14 (332.2 mg, 96%) as a pale yellow oil: ^1H NMR (600 MHz, CDCl_3) δ 13.8 (1H, brs), 5.21–5.19 (1H, m), 3.79 (2H, s), 3.72 (3H, s), 3.27 (2H, d, J = 6.0 Hz), 2.67 (3H, s), 2.38 (6H, s), 1.77 (3H, s), 1.70 (3H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 203.0, 166.3, 161.6, 160.7, 131.5, 123.5, 114.7, 107.4, 103.5, 62.6, 55.1, 44.2, 30.8, 25.7, 22.5, 17.9; HRFABMS m/z 307.1798 $[\text{M}]^+$ (calcd for $\text{C}_{17}\text{H}_{25}\text{NO}_4$, 307.1784).

1.1'-{(3-Methylbutane-1,1-diyl)bis[2,4-dihydroxy-6-methoxy-5-(3-methylbut-2-en-1-yl)-3,1-phenylene]}bis(ethanone) (15).—To a solution of 14 (32.1 mg, 0.10 mmol) in toluene (7.0 mL) were added 9 (32.3 mg, 0.19 mmol) and toluene (8.0 mL) at room temperature, and the mixture was refluxed for 1.0 h under N_2 . The mixture was cooled to room temperature and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:6–1:4–1:2) to afford the target 15 (14.2 mg, 32%) as a pale yellow solid, along with the recovered starting material 14 (10.3 mg, 32%): ^1H NMR (600 MHz, CDCl_3) δ 9.36 (2H, brs), 5.91 (1H, s), 5.21–5.19 (1H, m), 3.79 (2H, s), 3.71 (3H, s), 3.30 (2H, d, J = 5.4 Hz), 2.71 (3H, s), 2.69 (3H, s), 2.67 (3H, s), 1.78 (3H, s), 1.70 (3H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 204.2, 203.9, 160.3, 159.9, 132.1, 122.7, 116.2, 109.8, 108.3, 96.5, 62.7, 32.4, 30.6, 25.7, 23.0, 18.0, 15.9; HRFABMS m/z 431.1705 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{23}\text{H}_{27}\text{O}_8$, 431.1706).

1.1'-[Methylenebis(2,4-dihydroxy-6-methoxy-5-(3-methylbut-2-en-1-yl)-3,1-phenylene)]bis(ethanone) (16).—A solution of 14 (15.2 mg, 0.05 mmol) and 10 (14.9 mg, 0.06 mmol) in toluene (2.0 mL) was refluxed for 18 h. The mixture was cooled to room temperature and concentrated in vacuo. The residue was purified using preparative

TLC, with EtOAc/*n*-hexane (1:3) as eluent, to afford 16 (13.7 mg, 54%) as a pale yellow oil along with the recovered starting material 16 (6.2 mg, 42%): ¹H NMR (600 MHz, CDCl₃) δ 15.5 (2H, s), 9.58 (2H, brs), 5.21–5.19 (2H, m), 3.85 (2H, s), 3.71 (6H, s), 3.30 (4H, d, *J* = 6.6 Hz), 2.71 (6H, s), 1.77 (6H, s), 1.69 (6H, s); ¹³C NMR (150 MHz, CDCl₃) δ 204.0, 162.2, 160.3, 160.1, 131.9, 122.9, 116.3, 109.4, 108.2, 62.7, 30.6, 25.7, 23.0, 18.0, 16.4; HRFABMS *m/z* 513.2468 [M + H]⁺ (calcd for C₂₉H₃₇O₈, 513.2488).

Antiproliferative Activity Assay.

The compounds were evaluated for antiproliferative activity using the sulforhodamine B (SRB) assay, as described previously.¹¹ Briefly, test compounds were added to freshly harvested cells seeded on 96-well microtiter plates at a density of 4000–11 000 cells per well and cultured for 72 h. Cells were fixed in 10% trichloroacetic acid and then stained with 0.04% SRB. The absorbance of Tris base-solubilized protein-bound dye (10 mM) was measured at 515 nm using a microplate reader (ELx800, BioTek) operated by Gen5 software (BioTek). No less than three independent experiments in duplicate (*n* = 6) were conducted, and IC₅₀ data were calculated statistically (MS Excel). The cells were cultured in RPMI-1640 medium supplemented with 2 mM l-glutamine and 25 mM HEPES (Corning), containing 10% fetal bovine serum (Specialty Media), 100 µg/mL streptomycin, and 100 IU penicillin (Corning). KB-VIN stock cells, provided by Professor Y.-C. Cheng, Yale University, were maintained in the presence of 100 nM vincristine. The remaining human tumor cell lines in this study were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) or from ATCC (Manassas, VA, USA). The experimental control compound used was paclitaxel.

Supplementary Material

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ACKNOWLEDGMENTS

We appreciate critical comments, suggestions, and editing on the manuscript by Dr. S. L. Morris-Natschke (UNC-CH). This study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT KAKENHI, Japan), awarded to K.N.G. (grant number 25293024). This work was also supported by NIH grant CA177584 from the National Cancer Institute awarded to K.-H.L., as well as partial support from the Eshelman Institute for Innovation (Chapel Hill, NC, USA) awarded to M.G. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health (NIH), under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This project has also been supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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Figure 1.
Isolated compounds 1–8 from *Acronychia trifoliolata* and the structure of sideroxylonal C.

Figure 2. Selected HMBC correlations (arrows; black: in CDCl₃; red: in (CD₃)₂SO; green: in C₆D₆; blue: in (CD₃)₂CO), ¹H-¹H COSY connectivities (bold lines), and NOESY correlations (dotted arrows) for 1–3.

Figure 3.
Key NOESY correlations (blue arrows) for 1.

Figure 4.
Proposed biosynthetic 2*H*-pyran ring formation by Diels–Alder reaction.

Scheme 1.
Total Synthesis of Compound 3

Scheme 2.
Synthesis of Acetophenone Dimer Derivatives

Table 1.

 ^1H and ^{13}C NMR Spectroscopic Data of 1–3

position	1 (CDCl_3)		2 ($\text{DMSO}-d_6$)		3 (CDCl_3)
	δ_{H} (J in Hz) ^a	δ_{C}	δ_{H} (J in Hz) ^a	δ_{C}	δ_{H} (J in Hz) ^b
1		111.0		N.D. ^c	
2		160.4		159.0 ^d	
3		117.9		113.8 ^e	
4		161.6		161.5	
5		116.3		114.3	
6		158.7		159.0	
1'	3.29, m	27.1	5.24, brt (7.8)	26.7	4.70, t (7.2)
2'	1.33, m, 1.43, m	49.3	1.60, m, 2.09, m	40.7	2.15, m
3'	1.48, m	25.4	1.38, m	25.8	1.40, m
4'	0.87, d (6.0)	22.9	0.81, d (6.6)	22.1	0.89, brs
5'	0.91, d (6.0)	23.0	0.86, d (6.6)	23.1	0.89, brs
1''	3.22, t (6.0)	22.8	3.17, brs	22.4	3.30, brs
2''	5.05, m	122.5	5.09, brt (6.6)	123.5	5.18, brs
3''		132.0		130.3	
4''	1.50, brs	17.5	1.69, brs	17.8	1.77, brs
5''	1.57, brs	25.6	1.62, brs	25.4	1.70, brs
1'''		108.7		100.3	
2'''		161.1		161.6	
3'''		110.2		105.3	
4'''		161.4		161.8	
5'''		115.4		107.3 ^e	
6'''		161.3		161.2 ^d	
1''''	5.14, d (10.2)	79.1	2.97, m, 2.99, m	27.0	10.16, s
2''''	2.26, dt (10.2, 2.4)	51.9	4.71, dd (9.0, 9.0)	91.2	
3''''	1.71, m	28.4		70.0	
4''''	0.79, d (6.6)	16.6	1.11, d (2.4)	25.7	
5''''	0.96, d (7.2)	21.0	1.20, brs	25.6	
1'''''	3.34, dd (7.8, 6.6)	22.6			
2'''''	5.24, dd (7.8, 6.6)	123.0			
3'''''		131.9			
4'''''	1.79, brs	18.0			
5'''''	1.72, brs	25.7			
COCH_3 -1	2.73, s	31.4	2.58, s	30.8	2.73, s
COCH_3 -1		204.4		202.4	
COCH_3 -1'''	2.71, s	31.2	2.62, s	30.8	2.74, s
COCH_3 -1'''		203.9		203.3	

position	1 (CDCl ₃)		2 (DMSO- <i>d</i> ₆)		3 (CDCl ₃)
	δ_{H} (J in Hz) ^a	δ_{C}	δ_{H} (J in Hz) ^a	δ_{C}	δ_{H} (J in Hz) ^b
OCH ₃ -6'''	3.75, s	63.0	3.65, s	62.5	3.73, s
OCH ₃ -6'''	3.77, s	62.7			
OH	13.29, s (OH-2)		8.32, s		8.73, brs
OH	13.68, s (OH-2''')		9.37, brs		10.88, brs
OH	7.96, s (OH-4''')		10.69, brs		14.48, brs
OH			14.17, brs		16.11, brs
OH			14.67, brs		

^a Measured at 600 MHz.

^b Measured at 400 MHz.

^c Not detected.

^{d,e} Signals are interchangeable.

Table 2.

Antiproliferative Activity of the Isolated and Synthesized Compounds

compound	cell line ^a (IC ₅₀ μ M) ^b			
	A549	MDA-MB-231	KB	KB-VIN
2, 3, 6, 7	>10	>10	>10	>10
4	6.1	6.4	4.9	4.4
5	5.9	5.4	4.9	4.3
8	>10	10	6.0	6.5
12	4.9	5.8	5.2	5.0
13	>10	>10	7.6	8.4
15	5.6	6.9	5.2	5.1
16	6.4	5.9	5.5	5.5
paclitaxel (nM)	6.3	10.7	7.4	4531

^aA549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (identical to AV-3 as a cervical carcinoma HeLa derivative), KB-VIN (P-gp-overexpressing MDR subline of KB).

^bAntiproliferative activity expressed as IC₅₀ values for each cell line, the concentration of compound that caused 50% reduction relative to untreated cells determined by the SRB assay.

^cNot determined.