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Total Synthesis and in Vitro Anti-Tumor-Promoting Activities of Racemic Acetophenone Monomers from *Acronychia trifoliolata*

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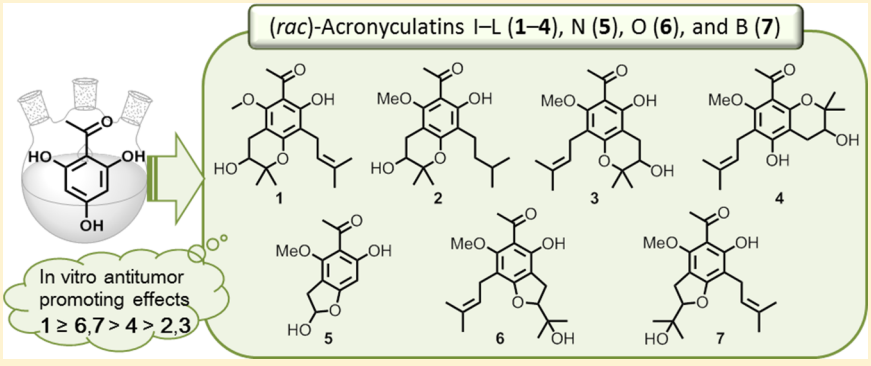
Supporting Information



ABSTRACT: Six acetophenone derivatives, acronyculatins I (**1**), J (**2**), K (**3**), L (**4**), N (**5**), and O (**6**), were recently isolated from *Acronychia trifoliolata*, and the structure of the known acronyculatin B (**7**) was revised. Because of the limited quantities of isolated products as well as their structure similarity, racemic acronyculatins I–L, N, O, and B (**1**–**7**) were synthesized to confirm their structures and to obtain sufficient material for biological evaluation. Trihydroxyacetophenone was converted to the target compounds by various sequences of hydroxy group protection, allylation or prenylation, and epoxidation followed by cyclization. *C*-Prenylations were carried out by direct addition of a prenyl group or through 1,3- or 3,3-sigmatropic rearrangement. The synthesized racemic compounds were evaluated in an anti-tumor-promoting assay using the Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells. All tested compounds significantly inhibited EBV-EA activation. Especially, racemic acronyculatin I (**1**) displayed the most potent inhibitory effects, with an IC_{50} value of 7.3 μM.

Prenylated acetophenones are mainly distributed in specific genera of the Rutaceae, such as *Acradenia*, *Bosistoa*, *Melicope*, *Medicosma*, and *Acronychia*.[1] In rare cases, they are found in the root bark of *Derris indica*[2] and Brazilian propolis.[3] More than 50 prenylated acetophenones have been isolated from the above species.[1] The prenyl group(s) is sometimes cyclized with a neighboring phenolic oxygen to form a pyran or furan ring. Interesting dimeric acetophenones, such as acrovestone, acropyrone, and acropyraonols, are found only in *Acronychia*.[4−8]

Acronychia trifoliolata Zoll. & Moritzi is distributed from Java and Christmas Island to the Solomon Islands, and only one phytochemical study has been reported on this species.[7] In the course of the discovery of unknown bioactive natural products from rainforest plants, six new acetophenone monomers, named acronyculatins I (**1**), J (**2**), K (**3**), L (**4**), M, N (**5**), and O (**6**), were isolated from a 1:1 CH_3OH/CH_2Cl_2 extract of this plant.[9] In addition, the structure of acronyculatin B (**7**), originally identified by Su et al.,[10] was revised to be 1-[6-hydroxy-2-(2-hydroxypropan-2-yl)-4-methoxy-7-(3-methylbut-2-en-1-yl)-2,3-dihydrobenzofuran-5-yl]ethan-1-one based on

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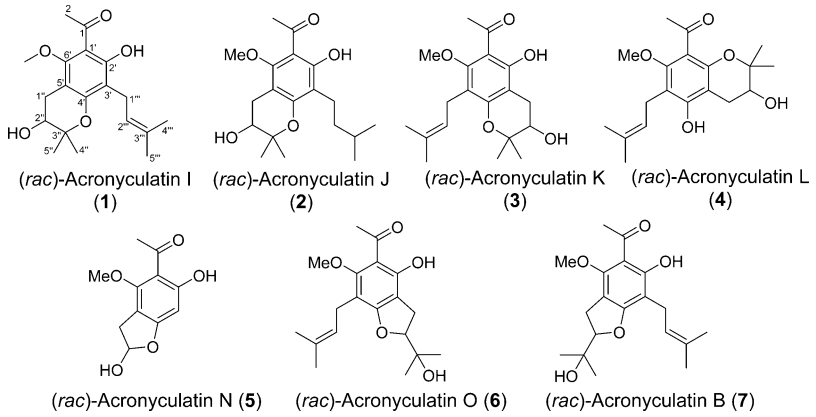
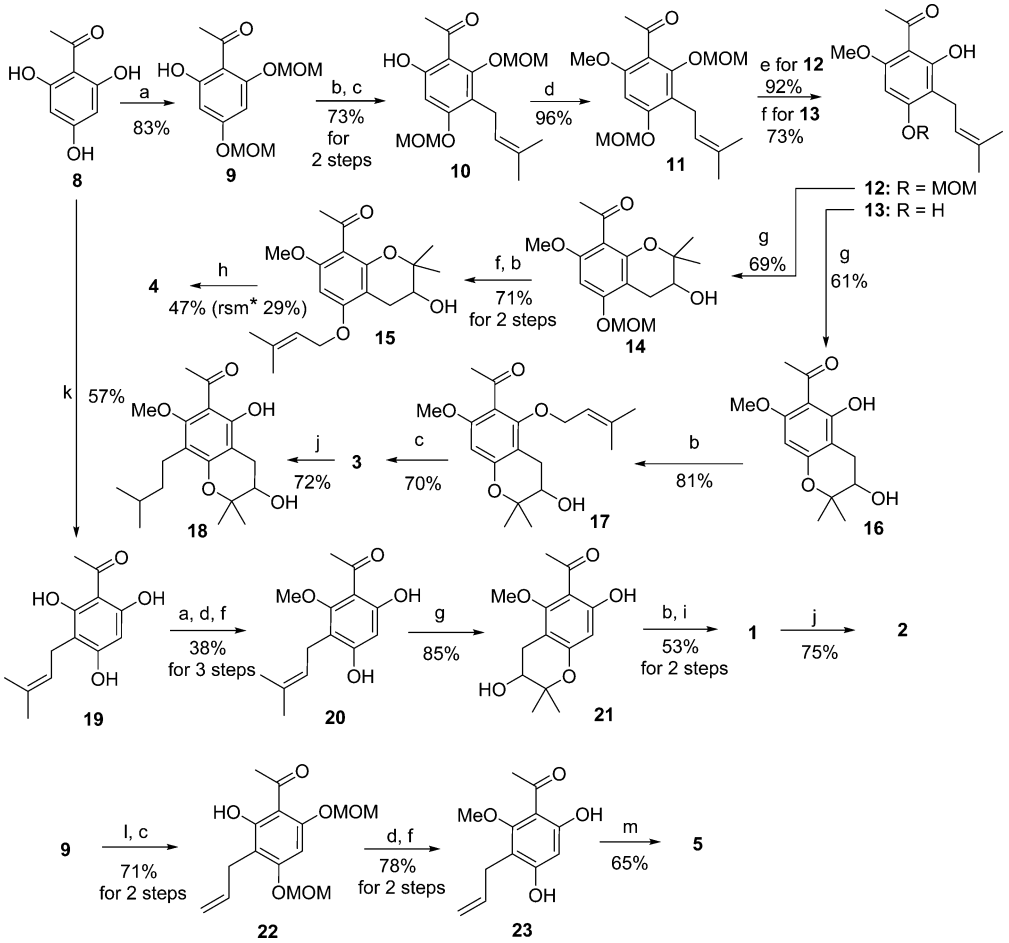


Scheme 1. Total Synthesis of Racemic Acetophenone Monomers 1−5[a]



[a]Conditions: (a) MOMCl, DIPEA, CH_2Cl_2, rt; (b) prenyl-Br, K_2CO_3, acetone, reflux; (c) $PhNEt_2$, microwave, 210 °C; (d) MeI, K_2CO_3, DMF, reflux; (e) 3 N HCl/MeOH (1:10), reflux; (f) 3 N HCl/MeOH (1:5), reflux; (g) mCPBA, CH_2Cl_2, rt, 20 min, then montmorillonite K10 (M-K10), 30 min; (h) M-K10, CH_2Cl_2, microwave, 60 °C; (i) M-K10, CH_2Cl_2, 0 °C to rt; (j) Pd/C, H_2, EtOH, rt; (k) prenyl-Br, DBU, THF, rt; (l) allyl-Br, K_2CO_3, acetone, reflux; (m) 2% OsO_4, $NaIO_4$, 1,4-dioxane, rt. *rsm: recovery of starting material.

extensive NMR studies. Because the isolation was performed on a limited amount (4.9 g) of extract provided by the U.S. National Cancer Institute (NCI), and no more material was available, small quantities of each compound were obtained. However, all isolated compounds were successfully identified using various NMR, HRMS, and IR techniques. Compounds **1**−**7** showed weak or no antiproliferative activity.[9] However, prior studies have indicated that acetophenone derivatives display potent inhibition of tumor-promoting activities.[11,12] In addition, the importance of a prenyl-like functional group was discussed in our previous reports.[13−16] Thus, prenylated acetophenones, such as the acronyculatins, could significantly inhibit the tumor-promoting activities. To confirm the structure elucidation as well as provide sufficient quantities of materials for further bioassays, the total syntheses of racemic acronyculatins **1**−**7** were performed. Herein, the synthesis details and evaluation of the anti-tumor-promoting activities are described.

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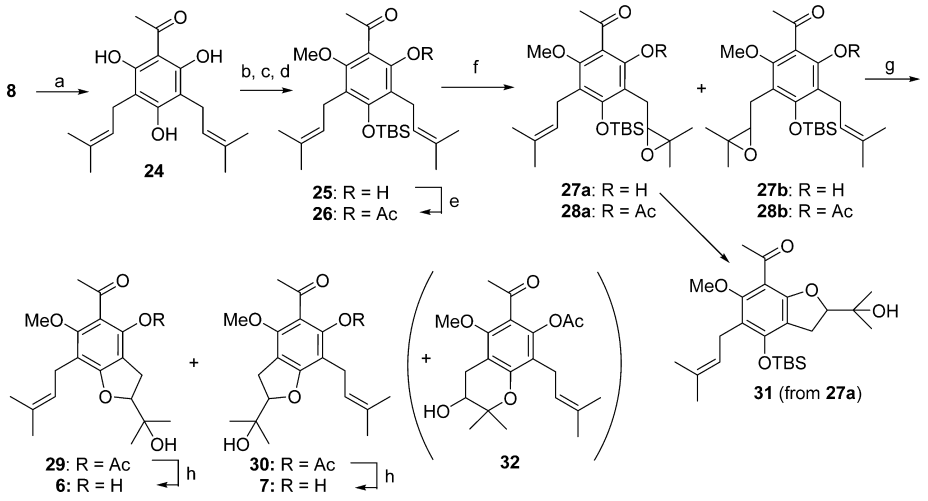
Scheme 2. Preparation of Racemic Acetophenone Monomers 6 and 7[a]



[a]Conditions: (a) prenyl-Br, 10% KOH_{aq}, CH_2Cl_2, rt, 1.5 h, 46%; (b) TBSCl, imidazole, DMAP, DMF, 85 °C, 45 min, 31%; (c) Me_2SO_4, NaH, THF, rt, 3.0 h, 91%; (d) TFA/H_2O, THF, rt, 1.0 h, 92%; (e) Ac_2O, DMAP, Py, rt, 1.0 h, 87%; (f) *m*CPBA, CH_2Cl_2, −70 °C, 30 min; (g) TBAF, HOAc, THF, 0 °C to rt, 1 h, 27% for 29, 64% for 30; (h) 0.1 M $Ba(OH)_2$, MeOH, 30 min, rt, 56% for **6**, 53% for 7.

RESULTS AND DISCUSSION

Since acetophloroglucinol is the core skeleton for all target compounds, 2,4,6-trihydroxyacetophenone (**8**) was selected as the starting material. Scheme 1 illustrates the synthesis of acetophenone monomers **1**−**5**. Two hydroxy groups of trihydroxyacetophenone (**8**) were first protected as methoxymethyl (MOM) ethers to provide **9**. Prenylation of the hydrogen-bonded phenolic group, followed by microwave-assisted *para*-Claisen rearrangement, produced **10**,[17] which was methylated with MeI to generate **11**. The selective removal of only one MOM protecting group was achieved successfully by controlled reaction conditions in 3 N HCl/MeOH (1:10 v/v) solution. Treatment of **12**[18] with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded an epoxide, and subsequent cyclization to the chromane **14** was catalyzed by montmorillonite K10 clay.[19] The removal of the remaining MOM group, prenylation of the phenolic group, and 1,3-rearrangement of the prenyl group using montmorillonite K10[20] gave racemic acronyculatin L (**4**). Racemic acronyculatin K (**3**) was synthesized similarly from **11**. Both MOM groups were removed by using more concentrated acidic conditions [3 N HCl/MeOH (1:5 v/v)] than those mentioned above for the selective removal of one MOM group. Two subsequent reactions (*m*CPBA, montmorillonite K10 clay) on the resulting **13**[18,21] produced only **16**, in which cyclization had occurred via the non-hydrogen-bonded hydroxy group. The *O*-prenylation and microwave-assisted *para*-Claisen rearrangement of **16** gave racemic acronyculatin K (**3**). Catalytic reduction of **3** produced unnatural compound **18**, a regioisomer of **2**.

Compound **8** was treated with prenyl bromide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[22] to generate monoprenylated acetophenone **19**, which was converted to **20** by MOM protection, methylation, and deprotection of MOM groups. Dihydroxyacetophenone **20** was treated with *m*CPBA and montmorillonite K10 clay to afford **21**. Prenylation of the phenolic group and subsequent 1,3-rearrangement gave racemic acronyculatin I (**1**), which was converted to racemic acronyculatin J (**2**) by catalytic reduction. Racemic acronyculatin M (**5**) was prepared from di-MOM ether **9**. *O*-Allylation of the phenolic group and subsequent microwave-assisted 3,3-sigmatropic rearrangement of the allyl ether produced **22**. Deprotection of both MOM groups gave **23**. Oxidative cleavage of the terminal olefinic bond was followed by spontaneous hemiacetalization to provide **5**.

As shown in Scheme 2, the prenylation of trihydroxyacetophenone **8** in the presence of 10% KOH produced diprenylated acetophenone **24**, which was converted to **25** through a three-step sequence, i.e., disilylation using *tert*-butyldimethylsilyl chloride (TBSCl), methylation, and selective deprotection[23] of the TBS ether. Treatment of **25** with 1 molar equiv of mCPBA at low temperature resulted in nonselective monoepoxidation of the olefinic functional groups. The resulting two products were probably the dihydrobenzofuran **31**, which was spontaneously cyclized through **27a**, and epoxide **27b**. To avoid the unfavorable cyclization, the phenolic moiety on **25** was protected as the acetate to give **26**. The monoepoxidation of **26** under the above conditions afforded **28a** and **28b** as an inseparable ca. 1:3 mixture by ^1H NMR analysis. The removal of the TBS group with tetra-*n*-butylammonium fluoride (TBAF)/HOAc prompted cyclization to the separable dihydrobenzofurans **29** and **30** in 27% and 64% yield, respectively. Under the same conditions, no chromane type of product was obtained, while the use of KF/18-crown-6/TFA at −10 °C generated **32** as a minor product and dihydrobenzofurans **29** and **30**. Hydrolysis of the acetoxy group of **29** and **30** by using $Ba(OH)_2$ generated racemic acronyculatins O (**6**) and B (**7**) in 56% and 53% yield, respectively.

The structures of the compounds were defined via HRMS and NMR data and were consistent with the assigned structures of the compounds[9] isolated from *A. trifoliolata* Zoll. & Moritzi.

As mentioned above, previous studies have indicated that prenyl-like structures and acetophenone derivatives tend to show potent effects on inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-mediated tumor-promoting activity.[11−16] Thus, compounds **1**−**7**, **18**, **29**, and **30** were evaluated for cancer chemopreventive activity by means of the Epstein−Barr virus early antigen (EBV-EA) activation stimulated by TPA in Raji cells. It has been proven that inhibitory effects on EBV-EA activation correlate well with anti-tumor-promoting activity in

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Table 3. Relative Ratio[a] of EBV-EA Activation with Respect to Positive Control (100%)

	percentage EBV-EA positive cells				
	concentration (mol ratio/TPA[b])				
	1000	500	100	10	IC_{50} (μM)[c]
1	0.0 ± 0.3 (60)[d]	26.5 ± 1.4	65.5 ± 2.5	92.4 ± 0.5	7.3
2	4.9 ± 0.2 (60)	31.5 ± 1.4	76.0 ± 2.6	97.7 ± 0.6	9.3
3	3.0 ± 0.3 (60)	29.1 ± 1.4	72.5 ± 2.4	96.9 ± 0.5	8.9
4	1.5 ± 0.2 (60)	27.1 ± 1.1	68.2 ± 2.5	96.6 ± 0.5	8.4
5	10.8 ± 0.5 (60)	53.3 ± 1.6	77.9 ± 2.2	100 ± 0.3	15.7
6	0.0 ± 0.3 (60)	32.0 ± 1.5	75.5 ± 2.3	94.9 ± 0.4	8.0
7	0.0 ± 0.3 (60)	30.6 ± 1.5	74.3 ± 2.5	93.3 ± 0.4	7.7
18	0.0 ± 0.4 (60)	36.6 ± 1.5	78.8 ± 2.3	97.8 ± 0.3	8.1
29	7.0 ± 0.6 (60)	43.8 ± 1.2	71.0 ± 2.3	100 ± 0.3	15.0
30	5.3 ± 0.5 (60)	33.9 ± 1.5	78.6 ± 2.4	97.8 ± 0.5	9.9
curcumin	0.0 ± 0.4 (60)	21.1 ± 1.1	80.1 ± 2.4	100 ± 0.1	12.1

[a]Values represent percentages relative to the positive control value (100%). [b]TPA concentration is 32 nM. [c]The concentration of compound required to inhibit 50% of the positive control activated with 32 nM TPA. [d]Values in parentheses are viability percentages of Raji cells.

vivo, as was previously reported for several natural product derivatives, such as the analogues of dimethyl biphenyldicarboxylate,[13] betulinic acid,[15,24] and coumarins.[16,25]

As shown in Table 3, the tested compounds displayed low to moderate cytotoxicity, as shown by high viability (60%) of Raji cells, implying less than 40% growth inhibition, even at a high concentration of TPA (32 nmol, a compound/TPA molar ratio of 1000:1). Furthermore, all compounds significantly inhibited TPA-mediated EBV-EA activation. Racemic acronyculatin I (**1**) showed the most potent inhibitory activity, with 100% inhibition of EBV-EA activation at the highest concentration (1000 mol ratio/TPA) and 7.6% inhibition at the lowest tested concentration (10 mol ratio/TPA). The IC_{50} value was 7.3 μM. Among the phenolic compounds **1**−**7** and **18**, compound **5**, devoid of a prenyl group, clearly exhibited reduced activity. These data supported the previous observations that a prenyl or prenyl-like group plays an important role in anti-tumor-promoting effects. Interestingly, the position of functional groups slightly affected the activity in the case of compounds with a chromane skeleton (**1** vs **3** and **2** vs **18**), while no difference was observed between the two compounds with a dihydrobenzofuran skeleton (**6** vs **7**). In addition, with the latter two compounds, the absence of a phenolic group led to decreased activity (**6** vs **29** and **7** vs **30**).

In summary, seven racemic acronyculatins isolated from *A. trifoliolata* Zoll. & Moritzi. and the acetophenone monomer **18** were synthesized. The NMR spectra of the synthetic racemic acronyculatins B and I−O were identical to those of the natural products. Evaluation of anti-tumor-promoting activity revealed that all tested acetophenones significantly inhibited EBV-EA activation induced by TPA in Raji cells. Especially, racemic acronyculatin I (**1**) displayed the most potent activity.

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 $CHCl_3$. NMR spectra were acquired on JEOL JMN-ECA600 and JMN-ECS400 spectrometers with tetramethylsilane as internal standard, and chemical shifts are expressed as δ values. HRMS data were obtained on a JMS-SX102A (FAB) or JMS-T100TD (DART) mass spectrometer. Microwave irradiation experiments were carried out in a dedicated Biotage Initiator 2.5 microwave apparatus. Analytical and preparative TLC was carried out on precoated silica gel $60F_{254}$ and RP-$18F_{254}$ plates (0.25 or 0.50 mm thickness; Merck). MPLC was performed with silica gel and C_{18} cartridges (Biotage, Uppsala Sweden). Compounds **9**−**13**[16,17,20] and **19**[21] were obtained previously.

1-[3-Hydroxy-7-methoxy-5-(methoxymethoxy)-2,2-dimethylchroman-8-yl]ethanone (14). To a solution of **12** (27.4 mg, 0.09 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added 75% *m*-CPBA (25.2 mg, 0.11 mmol) at 0 °C, and the mixture was stirred for 20 min at room temperature. After consumption of **12** (TLC), montmorillonite K10 (27.3 mg) was added and stirring was continued for 30 min at room temperature. The mixture was filtered and washed with EtOAc. The organic layer was washed with saturated Na_2CO_3, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2) to afford **14** (20.0 mg, 69%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.33 (1H, s), 5.20 (2H, s), 3.79−3.76 (1H, m), 3.77 (3H, s), 3.49 (3H, s), 2.87 (1H, dd, J = 16.8, 5.4 Hz), 2.65 (1H, dd, J = 17.4, 6.0 Hz), 2.46 (3H, s), 1.75 (1H, d, J = 7.2 Hz), 1.33 (3H, s), 1.31 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.7, 157.2, 156.3, 151.1, 114.3, 101.6, 94.4, 90.9, 77.5, 69.1, 56.2, 56.0, 32.6, 26.1, 24.6, 21.8; HRMS (FAB) m/z 311.1499 $[M + H]^+$ (calcd for $C_{16}H_{23}O_6$, 311.1495).

1-(3,5-Dihydroxy-7-methoxy-2,2-dimethylchroman-8-yl)-ethanone. To a solution of **14** (20.0 mg, 0.06 mmol) in anhydrous MeOH (1.5 mL) was added 3 N HCl (0.3 mL), and the mixture was refluxed for 1.0 h under N_2. After cooling to room temperature, the mixture was stirred for 15 min. The reaction was quenched with H_2O and extracted with EtOAc (3 × 10 mL). 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 (1:1) to afford the deprotected compound (16.0 mg, 93%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.00 (1H, s), 5.28 (1H, brs), 3.82−3.80 (1H, m), 3.70 (3H, s), 2.84 (1H, dd, J = 16.8, 4.8 Hz), 2.62 (1H, dd, J = 18.0, 6.6 Hz), 2.47 (3H, s), 1.91 (1H, d, J = 6.0 Hz), 1.34 (3H, s), 1.32 (3H, s); ^{13}C NMR (150 MHz, acetone-d_6) δ 199.3, 156.8, 156.1, 151.6, 112.8, 100.7, 91.4, 77.5, 68.7, 55.1, 31.8, 26.0, 25.1, 19.4; HRMS (FAB) m/z 267.1227 $[M + H]^+$ (calcd for $C_{14}H_{19}O_5$, 267.1232).

1-[3-Hydroxy-7-methoxy-2,2-dimethyl-5-(3-methylbut-2-enyloxy)chroman-8-yl]ethanone (15). To a solution of 1-(3,5-dihydroxy-7-methoxy-2,2-dimethylchroman-8-yl)ethanone (10.9 mg, 0.04 mmol) and K_2CO_3 (23.0 mg, 0.17 mmol) in acetone (1.0 mL) was added prenyl bromide (0.07 mL, 0.06 mmol). The mixture was heated under reflux for 2.0 h under N_2. After cooling to room temperature, the mixture was filtered, washed with EtOAc, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2) to afford **15** (10.4 mg, 76%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.07 (1H, s), 5.46−5.44 (1H, m), 4.53 (2H, d, J = 6.6 Hz), 3.79 (3H, s), 3.79−3.76 (1H, m), 2.85 (1H, dd, J = 16.8, 5.4 Hz), 2.63 (1H, dd, J = 17.4, 6.0 Hz), 2.47 (3H, s),

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1.79 (3H, s), 1.75 (3H, s), 1.73 (1H, d, J = 7.2 Hz), 1.33 (3H, s), 1.30 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.7, 158.9, 156.5, 151.3, 138.0, 119.5, 113.3, 100.9, 89.0, 77.5, 69.1, 65.2, 56.0, 32.6, 26.1, 25.8, 24.6, 21.8, 18.3; HRMS (FAB) m/z 335.1860 $[M + H]^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858).

(*rac*)-Acronyculatin L (4). To a solution of **15** (10.4 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added montmorillonite K10 (10.3 mg) at 0 °C. The mixture was heated in a microwave instrument at 60 °C for 4.0 h. The mixture was filtered, washed with EtOAc, and concentrated in vacuo. The residue was purified using preparative TLC with EtOAc/*n*-hexane (3:5) to afford the target **4** [4.9 mg, 66% (based on recovery of starting material)] as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.74 (1H, s), 5.23−5.20 (1H, m), 3.80−3.78 (1H, m), 3.71 (3H, s), 3.35 (2H, d, J = 7.2 Hz), 2.87 (1H, dd, J = 16.8, 4.8 Hz), 2.65 (1H, dd, J = 16.8, 5.4 Hz), 2.50 (3H, s), 1.85 (3H, s), 1.79 (3H, s), 1.74 (1H, brs), 1.34 (3H, s), 1.32 (3H, s); ^{13}C NMR (600 MHz, $CDCl_3$) δ 202.2, 155.5, 154.5, 149.5, 136.3, 121.9, 118.2, 111.8, 103.6, 77.5, 69.1, 63.5, 32.7, 26.2, 25.9, 24.7, 22.8, 21.9, 18.0; HRMS (FAB) m/z 335.1850 $[M + H]^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858).

1-(3,5-Dihydroxy-7-methoxy-2,2-dimethylchroman-6-yl)-ethanone (16). To a solution of **13** (48.2 mg, 0.19 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was added 75% *m*-CPBA (53.2 mg, 0.23 mmol) at 0 °C, and the mixture was stirred for 20 min at room temperature. After complete consumption of **13** (TLC), montmorillonite K10 (48.3 mg) was added and the mixture was further stirred for 30 min at room temperature. The mixture was filtered and washed with EtOAc. The organic layer was washed with saturated Na_2CO_3(aq), H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2) to afford **16** (31.3 mg, 61%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.4 (1H, s), 5.90 (1H, s), 3.85−3.82 (1H, m), 3.83 (3H, s), 2.87 (1H, dd, J = 16.8, 4.8 Hz), 2.65 (1H, dd, J = 16.8, 5.4 Hz), 2.61 (3H, s), 1.61 (1H, d, J = 6.6 Hz), 1.39 (3H, s), 1.33 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.2, 165.2, 161.5, 159.5, 105.6, 99.3, 91.1, 78.4, 69.1, 55.4, 32.9, 25.4, 24.8, 22.2; HRMS (FAB) m/z 267.1241 $[M + H]^+$ (calcd for $C_{14}H_{19}O_5$, 267.1232).

1-[3-Hydroxy-7-methoxy-2,2-dimethyl-5-(3-methylbut-2-enyloxy)chroman-6-yl]ethanone (17). To a solution of **16** (27.9 mg, 0.10 mmol) and K_2CO_3 (58 mg, 0.42 mmol) in acetone (2.0 mL) was added prenyl bromide (0.02 mL, 0.15 mmol), and the mixture was heated at reflux temperature for 20 h under N_2. The mixture was cooled to room temperature, filtered, washed with EtOAc, and concentrated in vacuo. The residue was purified using preparative TLC with $CHCl_3$/MeOH (20:1) to afford **17** (28.3 mg, 81%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.21 (1H, s), 5.47−5.45 (1H, m), 4.35 (2H, d, J = 7.2 Hz), 3.81−3.79 (1H, m), 3.76 (3H, s), 2.92 (1H, dd, J = 16.8, 4.8 Hz), 2.70 (1H, dd, J = 16.8, 5.4 Hz), 2.50 (3H, s), 1.77 (3H, s), 1.67 (3H, s), 1.65 (1H, s), 1.36 (3H, s), 1.32 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.1, 156.5, 155.9, 155.2, 138.8, 119.8, 118.9, 105.4, 96.2, 91.1, 71.6, 69.3, 55.7, 32.6, 26.3, 25.8, 24.7, 22.0, 18.1; HRMS (FAB) m/z 335.1854 $[M + H]^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858).

(*rac*)-Acronyculatin K (3). A solution of **17** (26.4 mg, 0.08 mmol) in *N*,*N*-diethylaniline (0.5 mL) was heated in a microwave instrument at 210 °C for 1.0 h. After cooling to room temperature, the mixture was washed with aqueous 10% HCl, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:5) to afford **3** (18.5 mg, 70%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.6 (1H, s), 5.15−5.13 (1H, m), 3.85−3.83 (1H, m), 3.71 (3H, s), 3.26 (2H, t, J = 5.4 Hz,), 2.91 (1H, dd, J = 17.4, 5.4 Hz), 2.68 (1H, dd, J = 17.4, 6.0 Hz), 2.69 (3H, s), 1.77 (3H, s), 1.68 (3H, s), 1.62 (1H, d, J = 7.2 Hz), 1.37 (3H, s), 1.32 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.4, 162.1, 159.1, 158.1, 131.1, 123.2, 115.0, 108.6, 103.2, 78.2, 68.9, 62.7, 30.9, 25.8, 25.7, 24.8, 22.5, 22.1, 18.0; HRMS (FAB) m/z 335.1852 $[M + H]^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858).

1-(3,5-Dihydroxy-8-isopentyl-7-methoxy-2,2-dimethylchroman-6-yl)ethanone (18). To a solution of **3** (8.1 mg, 0.02 mmol) in EtOH (0.5 mL) was added Pd/C (3.3 mg). The reaction mixture was sealed and stirred at room temperature for 2.0 h under H_2. The mixture was filtered through Celite, washed with EtOAc, and concentrated in vacuo. The residue was purified using preparative TLC with $CHCl_3$/MeOH (30:1) to afford the target **18** (5.9 mg, 72%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.6 (1H, s), 3.84 (1H, q, J = 4.8 Hz), 3.72 (3H, s), 2.90 (1H, dd, J = 16.8, 5.4 Hz), 2.68 (1H, dd, J = 17.4, 6.0 Hz), 2.68 (3H, s), 2.55−2.51 (2H, m), 1.63−1.60 (2H, m), 1.40−1.37 (1H, m), 1.38 (3H, s), 1.33 (3H, s), 0.97 (3H, s), 0.95 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.4, 161.9, 159.1, 158.1, 116.3, 108.6, 103.1, 78.1, 68.9, 62.9, 39.2, 30.9, 28.4, 25.9, 24.9, 22.6, 22.6, 22.1, 21.3; HRMS (FAB) m/z 337.1975 $[M + H]^+$ (calcd for $C_{19}H_{29}O_5$, 337.2015).

1-[2-Hydroxy-4,6-bis(methoxymethoxy)-3-(3-methylbut-2-enyl)phenyl]ethanone. To a solution of **19** (167.5 mg, 0.71 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was added *N*,*N*-diisopropylethylamine (0.35 mL, 2.01 mmol) at 0 °C, and the mixture was stirred for 20 min under Ar. MOMCl (0.13 mL, 1.71 mmol) was added, and stirring at room temperature continued for 45 min. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 × 10 mL). 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 CH_2Cl_2/*n*-hexane (1:1) to afford the target di-MOM ether (149.2 mg, 65%) as a pale yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.8 (1H, s), 6.39 (1H, s), 5.25 (2H, s), 5.23 (2H, s), 5.21−5.18 (1H, m), 3.51 (3H, s), 3.47 (3H, s), 3.30 (2H, d, J = 7.2 Hz), 2.65 (3H, s), 1.78 (3H, s), 1.66 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.5, 163.5, 160.7, 158.8, 131.4, 122.5, 111.6, 106.9, 94.6, 93.9, 91.2, 56.7, 56.3, 33.2, 25.8, 21.6, 17.8; HRMS (FAB) m/z 325.1668 $[M + H]^+$ (calcd for $C_{17}H_{25}O_6$, 325.1651).

1-[2-Methoxy-4,6-bis(methoxymethoxy)-3-(3-methylbut-2-enyl)phenyl]ethanone. To a solution of di-MOM ether (132.6 mg, 0.41 mmol) in anhydrous DMF (5.0 mL) were added K_2CO_3 (360.0 mg, 2.61 mmol) and iodomethane (0.08 mL, 1.23 mmol). The mixture was heated at reflux temperature for 2.5 h under N_2. After cooling to room temperature, the reaction was quenched with H_2O (10.0 mL) and extracted with EtOAc (3 × 15 mL). 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 (1:5) to afford the methyl ether (104.6 mg, 76%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.70 (1H, s), 5.19 (2H, s), 5.17−5.14 (1H, m), 5.14 (2H, s), 3.72 (3H, s), 3.47 (3H, s), 3.46 (3H, s), 3.29 (2H, d, J = 7.2 Hz), 2.52 (3H, s), 1.76 (3H, s), 1.67 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.2, 157.2, 156.1, 153.0, 131.3, 123.0, 120.8, 118.3, 97.9, 95.1, 94.3, 63.1, 56.4, 56.2, 32.6, 25.7, 22.7, 17.8; HRMS (FAB) m/z 339.1777 $[M + H]^+$ (calcd for $C_{18}H_{27}O_6$, 339.1808).

1-[4,6-Dihydroxy-2-methoxy-3-(3-methylbut-2-enyl)-phenyl]ethanone (20). To a solution of 1-[2-methoxy-4,6-bis-(methoxymethoxy)-3-(3-methylbut-2-enyl)phenyl]ethanone (54.7 mg, 0.16 mmol) in anhydrous MeOH (3.0 mL) was added 3 N HCl (0.6 mL), and the mixture was heated at reflux temperature for 1.5 h under N_2. The mixture was cooled to room temperature and stirred for 15 min, quenched with H_2O (10.0 mL), and extracted with EtOAc (3 × 15 mL). 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 (1:6) to afford the target **20** (31.2 mg, 77%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.2 (1H, s), 6.53 (1H, s), 6.22 (1H, s), 5.23−5.21 (1H, m), 3.74 (3H, s), 3.35 (2H, d, J = 7.2 Hz), 2.69 (3H, s), 1.82 (3H, s), 1.75 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.7, 164.1, 162.6, 161.4, 135.0, 121.9, 113.2, 109.5, 100.5, 62.8, 31.0, 25.8, 22.7, 18.0; HRMS (FAB) m/z 251.1290 $[M + H]^+$ (calcd for $C_{14}H_{19}O_4$, 251.1283).

1-(3,7-Dihydroxy-5-methoxy-2,2-dimethylchroman-6-yl)-ethanone (21). To a solution of **20** (29.2 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was added 75% *m*-CPBA (31.7 mg, 0.14 mmol) at 0 °C, and the mixture was stirred for 20 min at room temperature. After consumption of **20** (TLC), montmorillonite K10 (29.2 mg) was added and the mixture was stirred for 30 min at room temperature. The mixture was filtered and washed with EtOAc. The organic layer was washed with saturated Na_2CO_3, H_2O, and brine,

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dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2) to afford **21** (26.4 mg, 85%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.0 (1H, s), 6.20 (1H, s), 3.83−3.82 (1H, m), 3.78 (3H, s), 2.95 (1H, dd, J = 16.8, 5.4 Hz), 2.71 (1H, dd, J = 16.8, 6.6 Hz), 2.67 (3H, s), 1.99 (1H, d, J = 4.2 Hz), 1.37 (3H, s), 1.36 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.2, 163.7, 162.1, 160.2, 109.7, 104.9, 101.2, 78.1, 69.1, 61.5, 31.1, 26.0, 25.1, 21.8; HRMS (FAB) m/z 267.1228 [M + H]$^+$ (calcd for $C_{14}H_{19}O_5$, 267.1232).

1-[3-Hydroxy-5-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyloxy)chroman-6-yl]ethanone. To a solution of **21** (28.3 mg, 0.11 mmol) and K_2CO_3 (59.7 mg, 0.43 mmol) in acetone (2.0 mL) was added prenyl bromide (0.02 mL, 0.17 mmol), and the mixture was heated at reflux temperature for 12.5 h under N_2. The mixture was cooled to room temperature, filtered, washed with EtOAc, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc/*n*-hexane (1:2) to afford the product (34.3 mg, 97%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.21 (1H, s), 5.43−5.41 (1H, m), 4.45 (2H, d, J = 7.2 Hz), 3.82−3.79 (1H, m), 3.74 (3H, s), 2.91 (1H, dd, J = 16.8, 4.8 Hz), 2.68 (1H, dd, J = 13.8, 6.0 Hz), 2.50 (3H, s), 1.77 (3H, s), 1.69 (3H, s), 1.67 (1H, d, J = 6.6 Hz), 1.36 (3H, s), 1.32 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.1, 156.8, 156.1, 155.2, 137.9, 119.3, 118.8, 105.0, 101.3, 97.2, 69.1, 65.5, 62.1, 32.6, 26.0, 25.7, 24.8, 21.9, 18.2; HRMS (FAB) m/z 335.1870 [M + H]$^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858).

(*rac*)-Acronyculatin I (1). To a solution of 1-[3-hydroxy-5-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyloxy)chroman-6-yl]-ethanone (34.3 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added montmorillonite K10 (34.4 mg) at 0 °C, and the mixture was stirred for 3.0 h at room temperature. The mixture was filtered, washed with EtOAc, and concentrated in vacuo. The residue was purified using preparative TLC with EtOAc/*n*-hexane (1:1) to afford **1** (18.8 mg, 55%) as a colorless solid. The physical data (^{1}H NMR, ^{13}C NMR, HRMS) were essentially identical to the reported data for the natural product.[9]

(*rac*)-Acronyculatin J (2). To a solution of **1** (7.6 mg, 0.02 mmol) in EtOH (0.5 mL) was added Pd/C (3.6 mg). The reaction mixture was sealed and stirred at room temperature for 2.0 h under H_2. The mixture was filtered through Celite, washed with EtOAc, and concentrated in vacuo. The residue was purified using preparative TLC with $CHCl_3$/MeOH (30:1) to afford **2** (5.7 mg, 75%) as a colorless oil. The physical data (^{1}H NMR, ^{13}C NMR, HRMS) were essentially identical to the reported data for the natural product.[9]

1-[2-Allyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone. To a mixture of **9** (71.9 mg, 0.28 mmol) and K_2CO_3 (152.6 mg, 1.10 mmol) in acetone (3.0 mL) was added dropwise a solution of allyl bromide (0.07 mL, 0.83 mmol) in acetone (0.9 mL). The reaction mixture was stirred under reflux for 28 h. The mixture was cooled to room temperature, filtered, and evaporated in vacuo. The residue was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with column chromatography on silica gel to obtain the product (72.3 mg, 0.24 mmol, 86%) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.46 (1H, s, J = 2.0 Hz), 6.31 (1H, d, J = 2.0 Hz), 6.04−5.94 (1H, m), 5.40−5.35 and 5.28−5.25 (2H, each m), 5.14 (4H, d, J = 4.4 Hz), 4.53−4.51 (2H, m), 3.47 (6H, d, J = 5.6 Hz), 2.49 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.6, 159.6, 156.8, 155.3, 132.6, 117.7, 116.2, 96.2, 95.0, 94.8, 94.4, 69.4, 56.3, 56.2, 32.5; HRMS (FAB) m/z 297.1338 [M + H]$^+$ (calcd for $C_{15}H_{21}O_6$, 297.1355).

1-[2-Hydroxy-3-(2-propenyl)-4,6-bis(methoxymethoxy)-phenyl]ethanone (22). A solution of 1-[2-allyloxy-4,6-bis-(methoxymethoxy)phenyl]ethanone (104.6 mg, 0.35 mmol) in *N*,*N*-diethylaniline (0.5 mL) was irradiated in a microwave oven for 1 h at 210 °C. The mixture was cooled to room temperature and the reaction quenched with ice-cooled 15% aquoeus HCl. The residue was extracted three times with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with column chromatography on silica gel to obtain **22** (83.5 mg, 0.28 mmol, 83% based on recovery of starting material) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.9 (1H, s), 6.40 (1H, s), 5.98−5.90 (1H, m), 5.26 (2H, s), 5.23 (2H, s), 5.03−4.93 (2H, m), 3.52 (3H, s), 3.47 (3H, s), 3.38−3.36 (2H, m), 2.66 (3H, s).

1-[2-Methoxy-3-(2-propenyl)-4,6-bis(methoxymethoxy)-phenyl]ethanone. Iodomethane (0.023 mL, 0.37 mmol) was added to a solution of **22** (43.1 mg, 0.15 mmol) and K_2CO_3 (104.3 mg, 0.75 mmol) in DMF (2.0 mL), and the mixture was heated under reflux for 3 h. The mixture was cooled to room temperature and quenched with H_2O (20 mL). The residue was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified using column chromatography on silica gel to obtain the product (39.6 mg, 0.13 mmol, 87% based on recovery of starting material) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (1H, s), 6.00−5.91 (1H, m), 5.17 (2H, s), 5.14 (2H, s), 5.00−4.95 (2H, m), 3.12 (3H, s), 3.46 (3H, s), 3.46 (3H, s), 3.36−3.35 (2H, m), 2.51 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.2, 157.3, 156.3, 153.3, 136.8, 120.8, 116.4, 114.6, 97.8, 95.0, 94.4, 63.4, 56.4, 56.3, 32.6, 27.7; HRMS (FAB) m/z 311.1491 [M + H]$^+$ (calcd for $C_{16}H_{23}O_6$, 311.1495).

1-[2-Hydroxy-3-(2-propenyl)-4,6-dihydroxyphenyl]-ethanone (23). A solution of 1-[2-methoxy-3-(2-propenyl)-4,6-bis(methoxymethoxy)phenyl]ethanone (33.7 mg, 0.11 mmol) in MeOH (1.0 mL) was treated with HCl (0.09 mL, 0.18 mmol), and the mixture was heated under reflux for 7 h. The mixture was cooled to room temperature, quenched with H_2O (10 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with column chromatography on silica gel to obtain **23** (19.3 mg, 0.09 mmol, 90%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.2 (1H, s), 6.25 (1H, s), 6.09−6.02 (1H, m), 5.61 (1H, s), 5.21−5.18 and 5.15−5.14 (2H, each m), 3.75 (3H, s), 3.44−3.41 (2H, m), 2.70 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.6, 164.4, 161.9, 161.9, 136.2, 116.4, 111.0, 109.8, 100.6, 63.1, 31.0, 27.8.

(*rac*)-Acronyculatin N (5). A solution of **23** (4.3 mg, 0.02 mmol) in 1,4-dioxane (0.26 mL) was treated with 2% OsO_4/*tert*-BuOH (50 μL, 0.004 mmol), and the mixture was stirred in the dark at room temperature. After 30 min, 1.3% $NaIO_4/H_2O$ (0.8 mL, 0.05 mmol) was added dropwise. The reaction was quenched with H_2O (5 mL) and extracted with EtOAc (3 × 7 mL). The combined organic layers were washed with 20% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with column chromatography on silica gel to obtain **5** (3.1 mg, 0.013 mmol, 65%) as a colorless solid. The physical data (^{1}H NMR, ^{13}C NMR, HRMS) were essentially identical to the reported data for the natural product.[9]

1-[2,4-Bis(*tert*-butyldimethylsilyloxy)-6-hydroxy-3,5-bis(3-methylbut-2-enyl)phenyl]ethanone. To a solution of **24** (95.9 mg, 0.32 mmol) in anhydrous *N*,*N*-dimethylformamide (1.0 mL) were added 4-dimethylaminopyridine (3.8 mg, 0.03 mmol) and imidazole (68 mg, 1.00 mmol) at room temperature. The mixture was cooled to 0 °C, and then TBSCl (101.0 mg, 0.67 mmol) was added. The resulting mixture was allowed to warm to room temperature and was stirred for 45 min. The reaction mixture was adjusted to pH 1 by addition of 1 N HCl and extracted with EtOAc (3 × 10 mL). 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 CH_2Cl_2/*n*-hexane (1:6) to afford the title compound (51.7 mg, 31%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.0 (1H, s), 5.17−5.14 (2H, m), 3.26 (2H, d, J = 6.6 Hz), 3.20 (2H, d, J = 5.4 Hz), 2.58 (3H, s), 1.72 (3H, s), 1.68 (3H, s), 1.66 (3H, s), 1.64 (3H, s), 1.05 (9H, s), 1.01 (9H, s), 0.19 (6H, s), 0.04 (6H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 204.5, 158.9, 158.0, 153.3, 131.5, 131.1, 124.3, 124.3, 123.2, 117.3, 114.7, 112.6, 31.4, 26.1, 25.9, 25.7, 25.4, 24.4, 23.5, 18.8, 18.2, 18.1, 18.0, −3.03, −4.19; HRMS (FAB) m/z 533.3443 [M + H]$^+$ (calcd for $C_{30}H_{53}O_4Si_2$, 533.3482).

1-[2,4-Bis(*tert*-butyldimethylsilyloxy)-6-methoxy-3,5-bis(3-methylbut-2-enyl)phenyl]ethanone. A 60% NaH (10.0 mg) solution was washed with *n*-hexane (0.5 mL) and dissolved in anhydrous THF (0.5 mL). The mixture was cooled to 0 °C, and 1-

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[2,4-bis(*tert*-butyldimethylsilyloxy)-6-hydroxy-3,5-bis(3-methylbut-2-enyl)phenyl]ethanone (22.6 mg, 0.04 mmol) in anhydrous THF (0.5 mL) and dimethyl sulfate (0.02 mL, 0.19 mmol) were added under N_2. The mixture was allowed to warm to room temperature and stirred for 3.0 h. The reaction mixture was then adjusted to pH 8 by addition of saturated aqueous NH_4Cl and extracted with EtOAc (3 × 10 mL). 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 (1:20) to afford the product (21.1 mg, 91%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.17−5.13 (2H, m), 3.65 (3H, s), 3.24 (4H, m), 2.50 (3H, s), 1.69 (3H, s), 1.66 (3H, s), 1.65 (3H, s), 1.63 (3H, s), 1.00 (9H, s), 0.99 (9H, s), 0.21 (6H, s), 0.07 (6H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.7, 154.4, 154.0, 149.1, 131.1, 130.9, 123.9, 123.6, 121.7, 121.2, 62.7, 32.9, 26.1, 26.0, 25.6, 25.4, 24.8, 24.0, 18.7, 18.4, 18.1, 18.0, −3.20, −3.72; HRMS (FAB) *m*/*z* 547.3659 $[M + H]^+$ (calcd for $C_{30}H_{55}O_4Si_2$, 547.3639).

1-[4-(*tert*-Butyldimethylsilyloxy)-2-hydroxy-6-methoxy-3,5-bis(3-methylbut-2-enyl)phenyl]ethanone (25). To a solution of 1-[2,4-bis(*tert*-butyldimethylsilyloxy)-6-methoxy-3,5-bis(3-methylbut-2-enyl)phenyl]ethanone (14.8 mg, 0.03 mmol) in anhydrous THF (0.2 mL) were added TFA (0.1 mL) and H_2O (0.1 mL). The mixture was stirred at room temperature for 1.0 h. The reaction mixture was adjusted to pH 7 by addition of saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified using preparative TLC with EtOAc/*n*-hexane (1:12) to afford **25** (12.7 mg, 92%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.2 (1H, s), 5.17−5.14 (1H, m), 5.13−5.11 (1H, m), 3.66 (3H, s), 3.26 (4H, t, *J* = 6.6 Hz), 2.68 (3H, s), 1.72 (3H, s), 1.71 (3H, s), 1.67 (3H, s), 1.66 (3H, s), 1.00 (9H, s), 0.21 (6H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.9, 161.7, 159.3, 158.8, 131.8, 131.1, 123.7, 122.8, 118.4, 117.3, 110.4, 62.5, 31.0, 26.1, 25.7, 25.5, 23.8, 23.5, 18.9, 18.0, −3.05; HRMS (FAB) *m*/*z* 433.2733 $[M + H]^+$ (calcd for $C_{25}H_{41}O_4Si$, 433.2774).

2-Acetyl-5-(*tert*-butyldimethylsilyloxy)-3-methoxy-4,6-bis(3-methylbut-2-enyl)phenyl Acetate (26). To a solution of **25** (58.9 mg, 0.14 mmol) in pyridine (1.0 mL) were added DMAP (2.3 mg, 0.02 mmol) and Ac_2O (0.06 mL, 0.68 mmol), and the mixture was stirred at room temperature for 1.0 h. The reaction mixture was adjusted to pH 4 by addition of 1 N HCl and extracted with EtOAc (3 × 10 mL). 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 (1:10) to afford **26** (56.4 mg, 87%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.14−5.12 (1H, m), 5.02−4.99 (1H, m), 3.65 (3H, s), 3.30 (2H, d, *J* = 6.6 Hz), 3.19 (2H, d, *J* = 6.0 Hz), 2.52 (3H, s), 2.17 (3H, s), 1.71 (3H, s), 1.67 (3H, s), 1.67 (3H, s), 1.66 (3H, s), 1.00 (9H, s), 0.19 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5, 169.3, 155.6, 154.1, 144.9, 131.6, 131.5, 124.7, 123.0, 122.5, 122.4, 62.8, 31.3, 26.0, 25.6, 25.5, 24.5, 24.1, 20.8, 18.8, 18.0, 17.9, −3.11; HRMS (FAB) *m*/*z* 475.2862 $[M + H]^+$ (calcd for $C_{27}H_{43}O_5Si$, 475.2880).

2-Acetyl-5-[(*tert*-butyldimethylsilyl)oxy]-6-[(3,3-dimethyloxiran-2-yl)methyl]-3-methoxy-4-(3-methylbut-2-en-1-yl)phenyl Acetate (28a) and 2-Acetyl-5-[(*tert*-butyldimethylsilyl)oxy]-4-[(3,3-dimethyloxiran-2-yl)methyl]-3-methoxy-6-(3-methylbut-2-en-1-yl)phenyl Acetate (28b). To a solution of **26** (12.9 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added 75% *m*-CPBA (6.2 mg, 0.03 mmol) at −70 °C, and the mixture was stirred for 30 min. The reaction mixture was adjusted to pH 7 by addition of saturated aqueous Na_2CO_3 and extracted with CH_2Cl_2 (3 × 5 mL). 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 (1:5) to afford a mixture of the target compounds **28a** and **28b** [6.1 mg, 77% (based on recovery of starting material)] as a yellow oil.

5-Acetyl-2-(2-hydroxypropan-2-yl)-6-methoxy-7-(3-methylbut-2-en-1-yl)-2,3-dihydrobenzofuran-4-yl Acetate (29) and 5-Acetyl-2-(2-hydroxypropan-2-yl)-4-methoxy-7-(3-methylbut-2-en-1-yl)-2,3-dihydrobenzofuran-6-yl Acetate (30). To a mixture of **28a** and **28b** (6.1 mg, 0.01 mmol) in anhydrous THF (0.5 mL) were added HOAc (2.0 μL, 0.03 mmol) and TBAF (15.0 μL, 0.015 mmol), and the mixture was stirred at 0 °C for 10 min under Ar. To the mixture was added *p*-TsOH (2.4 mg, 0.01 mmol), and the mixture was stirred for 1.0 h. The mixture was adjusted to pH 7 by addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified using preparative TLC with EtOAc/*n*-hexane (1:2) to afford **29** (1.3 mg, 27%) and **30** (2.9 mg, 64%) as yellow oils.

5-Acetyl-2-(2-hydroxypropan-2-yl)-6-methoxy-7-(3-methylbut-2-enyl)-2,3-dihydrobenzofuran-4-yl Acetate (29). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.20−5.17 (1H, m), 4.68 (1H, t, *J* = 8.4 Hz), 3.70 (3H, s), 3.28 (2H, d, *J* = 7.8 Hz), 3.02−2.99 (2H, m), 2.51 (3H, s), 2.25 (3H, s), 1.76 (3H, s), 1.70 (3H, s), 1.30 (3H, s), 1.19 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 166.1, 160.8, 157.5, 153.2, 132.3, 121.7, 116.3, 115.3, 90.5, 77.2, 71.8, 63.4, 31.5, 28.4, 25.7, 25.6, 24.0, 23.0, 20.7, 17.8; HRMS (FAB) *m*/*z* 377.1975 $[M + H]^+$ (calcd for $C_{21}H_{29}O_6$, 377.1964).

5-Acetyl-2-(2-hydroxypropan-2-yl)-4-methoxy-7-(3-methylbut-2-enyl)-2,3-dihydrobenzofuran-6-yl Acetate (30). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.11−5.08 (1H, m), 4.66 (1H, t, *J* = 9.6 Hz), 3.86 (3H, s), 3.29−3.27 (2H, m), 3.16 (1H, dd, *J* = 15.0, 7.8 Hz), 3.10 (1H, dd, *J* = 14.4, 6.6 Hz) 2.46 (3H, s), 2.24 (3H, s), 1.72 (3H, s), 1.67 (3H, s), 1.35 (3H, s), 1.23 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.3, 169.5, 161.1, 152.8, 146.5, 132.2, 121.1, 114.0, 112.1, 90.1, 77.2, 71.6, 59.9, 31.8, 29.4, 26.0, 25.7, 24.3, 23.4, 20.7, 17.8; HRMS (FAB) *m*/*z* 377.1958 $[M + H]^+$ (calcd for $C_{21}H_{29}O_6$, 377.1964).

(*rac*)-Acronyculatin O (6). To a solution of **29** (2.0 mg, 0.005 mmol) in anhydrous MeOH (0.1 mL) was added $Ba(OH)_2$ (0.1 mL, 0.01 mmol, 0.1 M in MeOH), and the mixture was stirred for 30 min at room temperature. The mixture was directly purified using preparative TLC with $CHCl_3$/MeOH (20:1) to afford **6** (1.1 mg, 56%) as a colorless solid. The physical data (^{1}H NMR, ^{13}C NMR, HRMS) were essentially identical to the reported data for the natural product.[9]

(*rac*)-Acronyculatin B (7). To a solution of **30** (5.3 mg, 0.02 mmol) in anhydrous MeOH (0.2 mL) was added $Ba(OH)_2$ (0.2 mL, 0.02 mmol, 0.1 M in MeOH), and stirring continued for 30 min at room temperature. The mixture was directly purified using preparative TLC with $CHCl_3$/MeOH (20:1) to afford **7** (2.5 mg, 53%) as a colorless solid. The physical data (^{1}H NMR, ^{13}C NMR, HRMS) were essentially identical to the reported data for the natural product.[9]

In Vitro EBV-EA Activation Experiments. The anti-tumor-promoting activities of compounds were assessed using the EBV-EA activation assay in the presence of 32 pmol/mL TPA as described before.[26,27] The average EBV-EA induction of the test compound was determined as a ratio relative to the control. The viability of treated Raji cells was evaluated by trypan blue staining.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00646.

^{1}H NMR/^{13}C NMR for **1**−**7**, **14**−**18**, **20**−**23**, and **25**−**30**, the experimental procedures for known compounds **9**−**13**, **19**, and **24** (PDF)

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Notes

The authors declare no competing financial interest.

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