JOURNAL OF NATURAL PRODUCTS

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Cytotoxic Prenylated Acetophenone Dimers from *Acronychia pedunculata*

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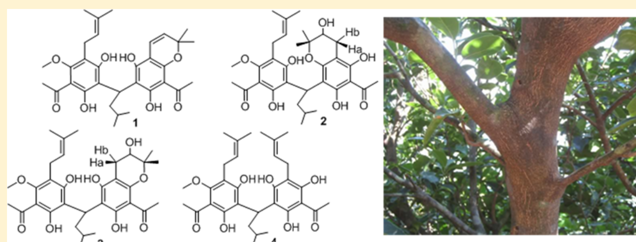
Supporting Information

ABSTRACT: Three new acetophenone dimers or *Acronychia*-type acetophenones, acropyrone (**1**), acropyranol A (**2**), and acropyranol B (**3**), were isolated from the trunk bark of *Acronychia pedunculata* and structurally characterized, together with four known acetophenone dimers, acrovestone (**4**), acrovestenol (**5**), acrofolione A (**6**), and acrofolione B (**7**), the acetophenone monomer acronyline (**8**), and four furoquinoline alkaloids. The chemical structures of the new isolated compounds were elucidated unambiguously by spectroscopic data analysis. The cytotoxic activities of the isolated acetophenone dimers were evaluated against the DU145 prostate and A2058 melanoma human cancer cell lines as well as the NHDF normal cell line. Acrovestone (**4**) and acrovestenol (**5**) exhibited substantial cytotoxicity, with IC_{50} values of 0.38 and 2.8 μM against A2058 melanoma cells as well as 0.93 and 2.7 μM against DU145 prostate cancer cells, respectively.



Acronychia is a genus of the plant family Rutaceae consisting of 42 species.[1] The roots, stems, leaves, and fruits of certain species in this genus have been used for centuries in eastern traditional medicine for the treatment of asthma, cough, diarrhea, itchy skin, pain, rheumatism, scales, sores, and ulcers and also for their antihemorrhagic, antipyretic, and aphrodisiac activities.[2] In particular, *Acronychia pedunculata* (L.) Miq. (= *Acronychia laurifolia* Bl.) is an evergreen tree distributed widely in rainforests of India, Sri Lanka, Indonesia, Malaysia, and southern mainland China.[1] According to previous studies, the most common secondary metabolites isolated from *A. pedunculata* are furoquinoline alkaloids[3−5] and prenylated acetophenone derivatives, of which the latter are mainly prenylated acetophenone monomers.[6−9] Special attention may be given to the prenylated acetophenone dimers since only a few have been reported and because these occur exclusively in the genus *Acronychia*, indicating their value as chemotaxonomic markers of the genus.[10]

Biological evaluation of extracts of *A. pedunculata* has shown significant antiplasmodial,[11] antibacterial,[12] and antifungal[13] activities as well as cytotoxic effects for several cancer cell lines.[11] Biological interest in prenylated acetophenones has focused on their antioxidant,[9] cytotoxic,[14] and anti-inflammatory[15] activities, while the acetophenone dimers have been assessed for cytotoxicity against numerous cancer cell lines, with acrovestone reported to exhibit significant cytotoxicity.[14,16] Despite their potential chemotaxonomic and biological importance, only a small number of acetophenone dimers have been isolated and biologically evaluated from the genus *Acronychia*.

In the present study, a phytochemical investigation is reported of an Et_2O extract from the trunk bark of *A. pedunculata* focused on the isolation of acetophenone dimers and the biological evaluation of these compounds. Specifically, three new *Acronychia*-type acetophenones were isolated and structurally identified, namely, acropyrone (**1**), acropyranol A (**2**), and acropyranol B (**3**). Moreover, four known acetophenone dimers, acrovestone[14] (**4**), acrovestenol[15] (**5**), acrofolione A[16] (**6**), and acrofolione B[16] (**7**), and the acetophenone monomer acronyline[17] (**8**) were obtained. All *Acronychia*-type acetophenones were tested for their cytotoxic

Received: December 30, 2011
Published: June 18, 2012

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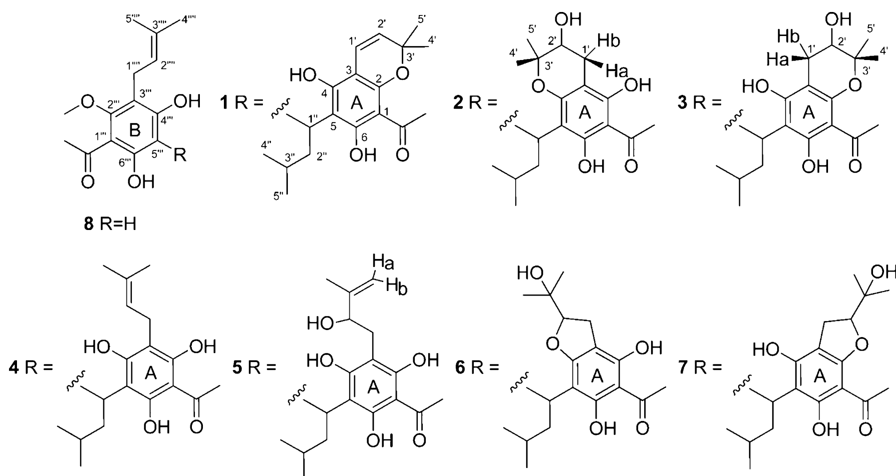

activities against the DU145 and A2058 human tumor cell lines as well as against the NHDF normal cell line, and compounds **4–6** demonstrated substantial cytotoxic activity. In addition, four furoquinoline alkaloids were isolated from the alkaloid fraction of the Et_2O extract and identified as dictamnine, pteleine, evolitrine, and kokusaginine.[18]

■ RESULTS AND DISCUSSION

Acetophenone dimers or *Acronychia*-type acetophenones may be considered as a particular group of acetophenones exhibiting specific structural features. They are polyhydroxylated, fully substituted derivatives consisting of two aromatic rings linked to an isopentyl chain. Apart from the hydroxy groups present, isoprenyl, acetyl, and methoxy units compose their common substituents, and additional rings derived from the isoprenyl moiety after cyclization have also been observed. Moreover, all the dimers reported so far as well as those described in the current study exhibit an extended degree of similarity and symmetry regarding the substitution of the two aromatic rings. For instance, in acrovestone (**4**), which represents a model compound for the *Acronychia*-type acetophenones, the two aromatic rings differ only in the presence of a methyl group. These structural peculiarities together with the presence of numerous rotamers complicate their structure elucidation using NMR spectroscopy. Specifically, the lack of signals in the aromatic region, signal overlapping, and poorly resolved and broad peaks in the NMR spectra complicate their identification and have resulted in inaccurate structural conclusions. In order to overcome these difficulties, all NMR experiments of the isolated compounds were recorded in the present work both at room temperature (25 °C, 298 K) and at 47 °C (320 K). The fast interconversion of rotamers due to alteration of temperature resulted in the improvement of peak shape of several proton signals with more comprehensive multiplicity, leading consequently to better resolution, facilitating the structure elucidation of acetophenone dimers considerably.

Compound **1** was isolated as an optically inactive yellowish oil and, therefore, characterized as a racemic mixture. The UV spectrum in MeOH showed characteristic absorption maxima for an acetophenone dimer at 214, 226, 289, and 333 (sh) nm.[14] Its molecular formula was deduced as $C_{32}H_{40}O_8$ from the APCI(+)-HRMS data, implying 13 degrees of unsaturation. The high-resolution mass spectrum revealed a pseudomolecular ion at m/z 553.2790 $[M + H]^+$ (calcd for 553.2796) as well as a fragment ion at m/z 319.1904, which is characteristic of all such isolated dimers (Figure MS1, Supporting Information). Based on its accurate mass (Δm = 0.0419 ppm), the proposed elemental composition, and the ring double-bond equivalent value, this ion corresponds to a fragment derived by the cleavage of the basic acetophenone skeleton at C-5. As an *Acronychia*-type acetophenone, compound **1** exhibited two fully substituted aromatic rings connected to an isopentyl chain. Despite the lack of signals in the aromatic region of the ^{1}H NMR spectrum, the presence of these rings was determined by characteristic signals corresponding to several deshielded quaternary carbons in the HMBC spectrum as well as from its accurately measured molecular mass and the degree of unsaturation evident. Following the common structural pattern of an *Acronychia*-type acetophenone, ring B appeared identical to known compounds, while ring A was assigned as 1-[5,7-dihydro-2,2-dimethyl-2*H*-1-benzopyran-8-yl]ethanone (IUPAC nomenclature). Thus, for all isolated dimers, the typical ring B substituents are a methoxy group in an *ortho* position to an isoprenyl and an acetyl group, in a *meta* position to two hydroxy groups, and in a *para* position to the characteristic isopentyl chain connecting the two aromatic rings.

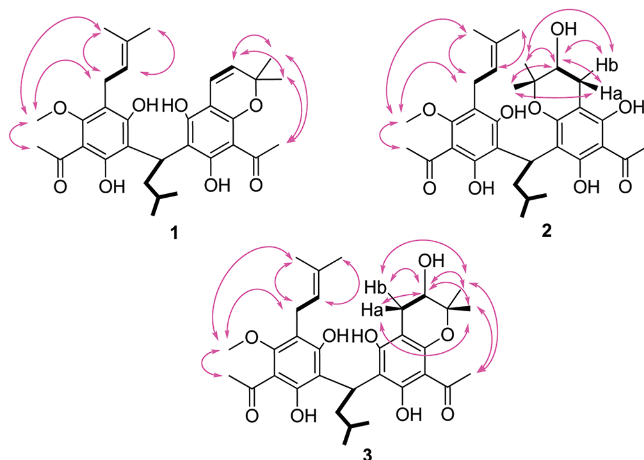

Figure 1. COSY (bold lines) and key NOE correlations (arrows) for compounds **1–3**.

Specifically, the ^{1}H NMR spectrum of **1** (Table 1) displayed signals ascribable to a characteristic isopentyl chain. The deshielded methine H-1″ signal between the two aromatic rings resonated at δ_H 4.73 as a triplet (J = 7.7 Hz), and the methylene H-2″ was observed at δ_H 2.17 as a multiplet, correlating with the corresponding carbon atoms at δ_C 28.3 and 39.2, respectively, as indicated from the HMQC spectrum. The methine H-3″

Table 1. NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds 1−3

	acropyrone (1)					acropyranol A (2)					acropyranol B (3)			
position	δ_H (*J* in Hz)[a]	δ_H (*J* in Hz)[b]	δ_C[b]	HMBC[b,c]	position	δ_H (*J* in Hz)[a]	δ_H (*J* in Hz)[b]	δ_C[b]	HMBC[b,c]	position	δ_H (*J* in Hz)[a]	δ_H (*J* in Hz)[b]	δ_C[a]	HMBC[a,c]
1			104.7		1			106.6		1			104.9	
2			155.6		2			162.4		2			154.8	
3			103.5		3			98.6		3			100.0	
4			158.6		4			155.1		4			161.9	
5			108.5[d,e]		5			106.6		5			108.7[d,e]	
6			160.7		6			159.6		6			161.6	
1′	6.66, d (10.1)	6.64, d (9.9)	117.0	2, 3, 4, 3′, 4′, 5′	1′a	2.94, dd (17.1/4.8)	2.92, dd (16.9/4.8)	25.7	2, 3, 4, 5, 2′, 3′	1′a	2.88, dd (17.2/5.0)	2.86, m	26.3	2, 3, 4, 2′, 3′
2′	5.43, d (10.1)	5.43, d (9.9)	124.9	2, 3, 3′, 4′, 5′	1′b	2.68, m	2.67, m		2′, 3′	1′b	2.62, m	2.63, m		2, 3, 4, 2′, 3′
3′			78.0		2′	3.86, t (4.8)	3.86, t (4.8)	68.7	3, 1′, 4′, 5′	2′	3.79, brs	3.78, m	68.7	
4′	1.48, s	1.47, brs	27.9	2′, 3′, 5′	3′			80.6		3′			78.4	
5′	1.48, s	1.47, brs	27.9	2′, 3′, 4′	4′	1.49, s	1.48, s	24.4	2′, 3′, 5′	4′	1.37, s	1.35, s	24.9	2′, 3′, 5′
1″	4.74, t (7.7)	4.73, t (7.7)	28.3	4, 5, 6, 2″, 3″, 4‴, 5‴, 6‴	5′	1.53, s	1.53, s	21.6	2′, 3′, 4′	5′	1.40, s	1.38, s	21.8	2′, 3′, 4′
2″	2.22, m	2.17, m	39.2	4″, 5″	1″	4.75, brs	4.74, brs	28.1	5, 6, 2″, 4‴, 5‴, 6‴	1″	4.75, t (7.5)	4.74, brs	28.2	5, 6, 2″, 3″, 5‴, 6‴
	2.14, m				2″a	2.31, m	2.32, m	40.0	5, 1″, 3″, 4″, 5″, 5‴	2″	2.16 brs	2.17, brs	39.4	
3″	1.43, m	1.41, m	26.8	2″, 4″, 5″	2″b	1.99, brs	1.89, m							
4″	0.88, d (5.2)	0.88, brs	22.5	2″, 3″, 5″	3″	1.41, m	1.38, m	26.8	2″, 4″, 5″	3″	1.42 m	1.40, m	27.0	1″, 2″, 4″, 5″
5″	0.88, d (5.2)	0.88, brs	22.5	2″, 3″, 4″	4″	0.90, d (6.6)	0.89, brs	22.3	2″, 3″, 5″	4″	0.88 brd (5.3)	0.88, brs	22.4	2″, 3″, 5″
1‴			108.0		5″	0.89, d (6.6)	0.89, brs	22.3	2″, 3″, 4″	5″	0.88 brd (5.3)	0.88, brs	22.4	2″, 3″, 4″
2‴			160.0		1‴			108.0		1‴			108.2	
3‴			116.4		2‴			160.1		2‴			160.2	
4‴			162.4		3‴			116.0		3‴			116.6	
5‴			112.7[d]		4‴			161.9		4‴			162.5	
6‴			160.7		5‴			112.5		5‴			112.9[d]	
1⁗	3.30, d (4.8)	3.29, brs	22.9	2‴, 3‴, 2⁗, 3⁗	6‴			159.6		6‴			161.6	
2⁗	5.20, tt (6.5/1.6)	5.19, brt (6.5)	123.0	3‴, 1⁗, 4⁗, 5⁗	1⁗	3.30, d (6.8)	3.29, brs	22.9	2‴, 3‴, 4‴, 2⁗, 3⁗	1⁗	3.30 brd	3.28, brd	23.1	2‴, 3‴, 4‴, 2⁗, 3⁗
3⁗			131.4		2⁗	5.16, tt (6.8/1.3)	5.16, t (6.6)	122.8	3‴, 1⁗, 4⁗, 5⁗	2⁗	5.20 t (6.4)	5.19, t (6.2)	123.2	3‴, 1⁗, 4⁗, 5⁗
4⁗	1.69, s	1.69, s	25.7	2⁗, 3⁗, 5⁗	3⁗			131.2		3⁗			131.4	
5⁗	1.77, s	1.76, s	17.9	2⁗, 3⁗, 4⁗	4⁗	1.67, s	1.66, s	25.7	2⁗, 3⁗, 5⁗	4⁗	1.68 s	1.68, s	25.5	3‴, 2⁗, 3⁗, 5⁗
MeO	3.71, s	3.71, s	62.6	2‴	5⁗	1.77, s	1.77, s	17.8	2⁗, 3⁗, 4⁗	5⁗	1.76 s	1.76, s	17.8	3‴, 2⁗, 3⁗, 4⁗
MeCO-1	2.68, s	2.68, s	32.8	1, MeCO-1	MeO	3.73, s	3.72, s	62.7	2‴	MeO	3.70 s	3.71, s	62.4	2‴
MeCO-1			202.9		MeCO-1	2.70, s	2.70, s	33.6	1, MeCO-1	MeCO-1	2.66 s	2.66, s	32.6	1, MeCO-1
MeCO-1‴	2.70, s	2.70, s	30.7	1‴, MeCO-1‴	MeCO-1			204.8		MeCO-1			203.6	
MeCO-1‴			203.9		MeCO-1‴	2.72, s	2.72, s	30.7	1‴, MeCO-1‴	MeCO-1‴	2.70 s	2.70, s	30.3	1‴, MeCO-1‴
OH-4		9.22/9.24, s[e]			MeCO-1‴			204.1		MeCO-1‴			204.1	
OH-4‴		10.08, s[e]			OH-2′		8.37, brs[e]			OH-2′				
OH-6		15.61/15.75, s[e]			OH-4		9.20, brs[e]			OH-4	9.24, s/9.29, d (13.2)[e]			
OH-6‴		15.92/16.10, s[e]			OH-4‴		9.86, brs[e]			OH-4‴	10.07, s/10.15, d (13.2)[e]			
					OH-6		14.04, s[e]			OH-6	15.59/15.72, s[e]			
					OH-6‴		15.65, s[e]			OH-6‴	16.05/16.22,s[e]			

[a]Spectra recorded at 47 °C. [b]Spectra recorded at 25 °C. [c]HMBC correlations from protons stated to the indicated carbon. [d,e]Assignments are interchangeable.

dx.doi.org/10.1021/np201007a | J. Nat. Prod. 2012, 75, 1270−1276

occurred as a multiplet at δ_H 1.41, while the protons of the two methyl groups, H-4″ and H-5″, resonated together as a broad peak at δ_H 0.88. The carbon at δ_C 26.8 was attributed to C-3″, and the signal at δ_C 22.5 to C-4″ and C-5″, due to their correlations with the corresponding protons in the HMQC spectrum. Moreover, the COSY experiment confirmed the sequence of the protons of the isopentyl chain. The position of the isopentyl chain between the two aromatic rings was determined by the cross-peak correlations of H-1″ with the downfield-shifted quaternary aromatic carbons at δ_C 158.6, 160.7, and 162.4 in the HMBC spectrum, which were assigned as C-4, C-6/6‴, and C-4‴, respectively.

The isoprenyl unit of **1** was deduced from the presence of a characteristic spin system consisting of a methylene proton at δ_H 3.29 that appeared as a broad singlet (H-1⁗) correlating with a carbon (HMQC spectrum) at δ_C 22.9. Also, an olefinic proton appeared as a broad triplet at δ_H 5.19 (J = 6.5 Hz, H-2⁗), correlating with a carbon at δ_C 123.0 along with two methyl groups at δ_H 1.69 (3H, s, H-4⁗) and 1.76 (3H, s, H-5⁗) correlating with carbon signals at δ_C 25.7 and 17.9, respectively. The HMBC spectrum revealed a cross-peak correlation between H-1⁗ and an olefinic quaternary carbon at δ_C 131.4 and was assigned therefore as C-3⁗. Moreover, useful NOE correlations were observed between H-1⁗ and H-5⁗ signals, as well as between those of H-2⁗ and H-4⁗, and were used to define the relative orientation of the two methyl groups. In the NOESY spectrum were also observed correlations between the protons of the methoxy group at C-2‴ at δ_H 3.71 (3H, s, CH_3O-2‴) and the H-1⁗ and H-5⁗ protons. The position of the isoprenyl unit on ring B was defined through the HMBC correlation of H-1⁗ with two downfield shifted quaternary carbons at δ_C 160.0 (^3J) and 162.4 (^3J) assigned as C-2‴ and C-4‴. Likewise, the correlation of the CH_3O-2‴ protons with C-2‴ (^3J) observed in the HMBC spectrum and the correlation of H-1″ with C-4‴ (^3J) and C-6‴ (^3J) revealed the positions of the ring B substituents. Finally, a NOE correlation of the methoxy protons CH_3O-2‴ with the protons at δ_H 2.70 (3H, s, CH_3CO-1‴) and a HMBC correlation of the CH_3CO-1‴ protons with C-1‴ (^3J) at δ_C 108.0 supported the presence of an acetyl group in an *ortho* position to the CH_3O-2‴.

An additional 2,2-dimethyl-2*H*-pyran ring attached to ring A in **1** was deduced by the two characteristic doublets at δ_H 6.64 (H-1′) and 5.43 (H-2′) in the ^{1}H NMR spectrum with a coupling constant of 9.9 Hz, typical of the olefinic protons of a pyran ring. Correlations of these protons with two carbon atoms at δ_C 117.0 and 124.9, respectively, were evident in the HMQC spectrum. Also characteristic were the signals of the H-4′ and H-5′ methyl groups that resonated at δ_H 1.47 as a broad singlet integrating for six protons and correlating with a carbon atom at δ_C 27.9 assigned as C-4′/C-5′. Moreover, the presence of a quaternary carbon at δ_C 78.0 correlating with H-1′ (^3J), H-2′ (^2J), H-4′ (^2J), and H-5′ (^2J) in the HMBC spectrum resulted in its assignment as C-3′. The assigment of the fusion of the ring to C-2/C-3 was confirmed unambiguously through NOE correlation of the protons of the methyl groups (H-4′ and H-5′) with the methyl protons of the acetyl group at δ_H 2.68 (3H, s, CH_3CO-1). Moreover, two pairs of singlets were observed at δ_H 15.92/16.10 and 15.61/15.75 in the ^{1}H NMR spectrum corresponding to the downfield shifted OH-6 and OH-6‴ signals, due to the presence of hydrogen bonds with the carbonyl group of the acetyl moieties CH_3CO-1 and CH_3CO-1‴, respectively. Furthermore, OH-4 and OH-4‴ resonated at δ_H 9.22/9.24 (two singlets) and at δ_H 10.08 (singlet), respectively. It is important to note that the signals corresponding to the hydroxy groups were observed only in the ^{1}H NMR spectrum recorded at 25 °C. Therefore, compound **1** could be proposed structurally as 1-[6-[1-[3-acetyl-2,6-dihydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl]-3-methylbutyl]-5,7-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran-8-yl]-ethanone and was given the trivial name acropyrone.

Compound **2** was also obtained as a yellowish optically inactive oil. Its APCI(+)-HRMS provided the molecular formula $C_{32}H_{42}O_9$ based on the pseudomolecular ion $[M + Na]^+$ at m/z 593.2725 (calcd for 593.2721) and the typical fragment of *Acronychia*-type acetophenones at m/z 319.1907, implying 12 degrees of unsaturation (Figure MS2, Supporting Information). UV absorption maxima in MeOH at 210, 229, 292, and 340 (sh) nm revealed the presence of a typical *Acronychia*-type acetophenone structure.

The ^{1}H NMR data (Table 1) were closely related to those of **1**. Apart from the signals corresponding to the constant part of the molecule (ring B), an additional 3-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-pyrano ring was observed at ring A, characterized by a substitution pattern that is now being reported for the first time among *Acronychia*-type acetophenone derivatives. Specifically, in the ^{1}H NMR spectrum, signals corresponding to a methylene group were observed at δ_H 2.92 as a doublet of doublets (J = 16.9/4.8 Hz, H-1′a) and at δ_H 2.67 as a multiplet (H-1′b). The signal of a methine group (H-2′) resonated at δ_H 3.86 as a triplet with a coupling constant of 4.8 Hz also was observed. Finally, the protons of the H-4′ and H-5′ methyl groups were observed as two singlets at δ_H 1.48 and 1.53, respectively. The correlations in the HMQC spectrum of H-1′a/H-1′b and H-2′ with the carbons at δ_C 25.7 and 68.7, respectively, confirmed the position of the additional OH group at C-2′. The HMBC correlations of H-1′a/H-1′b (^3J) and H-2′ (^2J) with a quaternary carbon at δ_C 80.6 led to the assignment of this carbon as C-3′. Carbon atoms C-4′ and C-5′ at δ_C 24.4 and 21.6, respectively, were defined through cross-peak correlations observed between them and the H-4′ and H-5′ methyl protons, in the HMQC spectrum. Furthermore, the positions of the methyl groups at C-4′ and C-5′ were determined from their HMBC correlations with C-2′ (^3J) and C-3′ (^2J). Similarly to **1**, the OH-6‴ and OH-6 groups were observed significantly deshielded as two single peaks at δ_H 14.04 and 15.65 in the ^{1}H NMR spectrum, while the OH-2′, OH-4, and OH-4‴ signals were observed at δ_H 8.37, 9.20, and 9.86 as broad singlet peaks, respectively. Finally, the 3-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-pyrano ring was demonstrated to be fused at the C-3/C-4 positions, as there was no NOE correlations of the protons of the methyl groups (H-4′ and H-5′) with the methyl protons of CH_3CO-1 that resonated at δ_H 2.70 (3H, s). Due to the small isolated quantity of **2**, the absolute configurations of C-2′ and C-1″ could not be defined. However, useful NOE correlations of the protons of the methyl groups (H-4′ and H-5′) with H-1′a and H-1′b, respectively, determined their relative orientation. Compound **2** (acropyranol A) was identified therefore as 1-[8-[1-[3-acetyl-2,6-dihydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl]-3-methylbutyl]-3,5,7-trihydroxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-6-yl]ethanone.

Compound **3** was isolated as an optically inactive yellowish oil, and its UV absorption maxima (MeOH), observed at 209, 231, 298, and 329 (sh) nm, were typical for an *Acronychia*-type acetophenone. Its molecular formula was determined as $C_{32}H_{42}O_9$, implying 12 degrees of unsaturation, based on the APCI(+)-HRMS data, such as its pseudomolecular ion $[M + H]^+$

at m/z 571.2897 (calcd for 571.2902) and the typical fragment of *Acronychia*-type acetophenones at m/z 319.1905 (Figure MS3, Supporting Information). In exhibiting a different retention time at the HPLC-DAD chromatogram (Figure H1, Supporting Information) and the same mass spectrometric data as **2**, compound **3** was concluded to be a structural isomer of **2**.

Indeed, the NMR data (Table 1) of **3** were found to be very closely related to those of **2**. As for compound **2**, all the signals corresponding to the typically substituted ring B were detected and determined from the NMR spectra (^{1}H NMR, HSQC, HMBC). Moreover, characteristic signals corresponding to the 3-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-pyrano ring were also observed. However, differences in the chemical shifts of specific protons (^{1}H NMR) and carbons (HMBC) were evident. In particular, the methylene protons of the additional ring resonated at δ_H 2.86 (1H, m, H-1′a) and 2.63 (1H, m, H-1′b) and correlated to a carbon signal at δ_C 26.3, while the protons of the methine H-2′ appeared at δ_H 3.78 (1H, m) and correlated to a carbon at δ_C 68.7 (HMQC). Finally, the signals of the characteristic H-4′ and H-5′ methyl protons were observed at δ_H 1.35 (3H, s) and 1.38 (3H, s) and correlated with their corresponding carbon atoms at δ_C 24.9 and 21.8 in the HMQC spectrum. Characteristic also were the HMBC correlations of H-1′b (^3J), H-4′ (^2J), and H-5′ (^2J) with a quaternary carbon at δ_C 78.4, leading to the assignment of this carbon as C-3′. Similar to compound **1**, hydroxy group signals for OH-6 and OH-6‴ were observed as two pairs of downfield shifted singlets at δ_H 15.59/15.72 and 16.05/16.22 in the ^{1}H NMR spectrum, while signals for OH-4 and OH-4‴ resonated at δ_H 9.24/9.29 and 10.07/10.15, respectively. It is important to note the difference in chemical shifts of the hydroxy groups between compounds **1**, **3**, and **2** (^{1}H NMR). Specifically, OH-6 and OH-6‴ of compounds **1** and **3** appeared more shielded at δ_H 15.5−16.5 as two pairs of double peaks, while in compound **2** they are observed at δ_H 14.04 and 15.65 as singlets. This difference indicates the opposite fusion pattern of the additional ring of **1** and **3** when compared to **2**. Moreover, NOE correlations between protons H-4′, H-5′ of the methyl groups and the protons of the CH_3CO-1 group at δ_H 2.66 (3H, s) confirmed the position as well as the fusion of this ring at the C-2 and C-3 positions. The last correlation was absent in the NOESY spectrum of **2**, illustrating the different fusion profile. Similar to compound **2** and due to the small quantity of compound **3** isolated, the absolute configurations at C-2′ and C-1″ were not deduced, but the NOE correlations observed between the H-4′ and H-1′a protons as well as the H-5′ and H-1′b facilitated the determination of their relative orientation. Thus, compound **3** (acropyranol B) was assigned as 1-[6-[1-[3-acetyl-2,6-dihydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl]-3-methylbutyl]-3,5,7-trihydroxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-8-yl]ethanone.

Previously, compounds **6** and **7** have been reported in a phytochemical study of *Acronychia trifoliolata*.[16] However, their structural differentiation was not evident regarding the position of the additional furan ring. More specifically, similar to acropyranol A (**2**) and acropyranol B (**3**), compounds **6** and **7** are structural isomers differing only at the fusion position of the 2-(2-hydroxypropan-2-yl)-2,3-dihydro-1-furan ring occurring at either C-2/C-3 or C-4/C-3 of ring A. According to the present study, the position of this ring could be deduced by the correlation of the protons of the methyl groups of the 2-(2-hydroxypropan-2-yl)-2,3-dihydro-1-furan ring with those of the acetyl group of the ring A, as observed in the NOESY spectrum. In particular, a NOE correlation was observed of the protons of the H-4′ and H-5′ methyl groups at δ_H 2.51 (3H, s) and 2.25 (3H, s) with the protons of the ring A acetyl group at δ_H 2.64 (3H, s, CH_3CO-1) in the spectrum of **7**, a correlation not observed in the analogous spectrum of **6** (Figures NS16 and NS17, Supporting Information). The same difference was evident from the NOESY spectra of compounds **2** and **3**, indicating the position of the hydroxypyrano ring (Figures NS10 and NS15, Supporting Information). Thus, this characteristic cross-peak correlation may be used as a diagnostic signal not only for the specific pair of isomers but generally for the determination of the position of the additional ring in *Acronychia*-type acetophenone derivatives.

The cytotoxicity of the isolated *Acronychia*-type acetophenones (**1**−**7**) was examined using an MTS assay against human DU145 prostate cancer and A2058 melanoma cells. Cell viability (%) was normalized to the vehicle control (Table B1, Supporting Information). All acetophenones tested inhibited differentially cell viabilities for both cell lines. Compounds **1**−**3** and **7** displayed relatively weak cytotoxicity, while compounds **4**−**6** showed substantial cytotoxicity for both cell lines, as shown in Table 2. Interestingly, compounds **4** and **5** exhibited the most potent activity, with IC_{50} values of 0.38 and 2.8 μM, among the compounds tested against A2058 melanoma cells (Table 2). Among the *Acronychia*-type acetophenones, compounds **4** and **5** were the most effective for DU145 cells, with IC_{50} values of 0.93 and 2.7 μM, respectively. All compounds were also assayed for their cytotoxicity against normal human dermal fibroblast (NHDF) cell line (Table 2). They were found to be inactive and exhibited IC_{50} values of >5 μM (Table 2), thereby suggesting these compounds may be selective to tumor cells. These data suggest also that the presence of a short aliphatic, hydrophobic chain such as an isoprenyl (**4**) or modified isoprenyl moiety (**5**) at the C-3 position of the ring A enhances cytotoxicity against both the tumor cell lines used. In contrast, the presence of an additional ring seems to reduce the cytotoxicity of the acetophenone dimers (**1**, **2**, **3**, and **7**) investigated. Moreover, the presence of an additional ring fused at the C-3 and C-4 positions (ring A) seems to enhance the cytotoxic activity compared to their isomers having the additional ring fused at the C-2 and C-3 positions (**2** vs **3** and **6** vs **7**). It is worth noting the different activity profile of the two isomers **6** and **7** against the two cancer cell lines used. This difference indicates that the relative position of the additional ring on the basic *Acronychia*-type acetophenone skeleton contributes significantly to the cytotoxic activity.

Table 2. IC_{50} Values of Compounds 4−6 against Two Human Tumor and a Normal Cell Line

	IC_{50} value (μM)[a]		
compound	DU145	A2058	NHDF
4	0.93 ± 0.07[b]	0.38 ± 0.04	>5.0
5	2.7 ± 0.5	2.8 ± 0.3	>5.0
6	>5.0	4.2 ± 0.6	>5.0
sorafenib	5.1 ± 0.7	3.8 ± 0.9	

[a]Cells were treated with compounds in a dose-dependent manner for 48 h,; then the MTS assay was used to assess cell viability. [b]Data are expressed as means ± SD.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. Nuclear magnetic

resonance (NMR) spectra were recorded on a Bruker Advance 600 MHz spectrometer using $CDCl_3$ (Aldrich) as solvent. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals (δ H 7.26/δ C 77.0). The 2D NMR experiments (COSY, HMQC, HSQC, HMBC, and NOESY) were performed using standard Bruker microprograms. APCI-HRMS were run on an Orbitrap Discovery (Thermo Finnigan, San Jose, CA, USA) spectrometer. Fast centrifugal partition chromatography (FCPC) was performed using a CPC Kromaton with a 1000 mL column and a Laboratory Alliance pump with a pressure safety limit of 50 bar. A manual sample injection valve was used to introduce the samples into the column, with the rotation adjusted at 800 rpm and the flow rate held at 20 mL/min. Analytical HPLC was performed on a Thermo Finnigan apparatus equipped with a PDA Spectra System UV6000LP using an Ascentis RP-8 C_8 (250 × 4.6 mm i.d.; 5 μm) (Discovery Supelco) column. Semipreparative HPLC was performed on a Thermo Finnigan apparatus equipped with a UV Spectral System UV2000 on an Ascentis RP-8 C_8 (250 × 10 mm i.d.; 5 μm) (Discovery Supelco) column.

Plant Material. The trunk bark of *Acronychia pedunculata* was collected in the dense rainforest of Mersing, Johore State, Malaysia, in April 1999. The plant was identified by botanist T. Leong Eng. A voucher specimen (KL-4882) has been deposited at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

HPLC Analysis. Chromatographic analysis of all extracts, FCPC fractions, and pure compounds was performed by HPLC-PDA. Volumes of 20 μL corresponding to 200 μg of extract, 100 μg of FCPC fractions, and 20 μg of pure compounds were injected. The mobile phase consisted of 2% CH_3COOH in H_2O and MeOH. The flow rate was 1 mL/min, and separation occurred at room temperature.

Extraction and Isolation. The dried and pulverized trunk bark of the plant (2.4 kg) was extracted with Et_2O (2 × 5 L of Et_2O, 48 h per extraction) at room temperature. After concentration under reduced pressure, 224.2 g of the first Et_2O residue was obtained, from which 15 g was initially fractionated by FCPC with a two-phase solvent system composed of *n*-heptane−ethyl acetate−methanol−water (10:1:10:1), using the organic phase first as mobile phase. The separation afforded 80 fractions of 50 mL each. After the collection of the 60 initial fractions, the apparatus was switched to the descending mode, and another 20 fractions were collected. Two fractions out of the eighty obtained contained in high purity the two major metabolites of the Et_2O extract, acrovestone (**4**, 243.2 mg; fraction 13) and acrofolione A (**6**, 471.8 mg; fraction 71), while the other metabolites were isolated as mixtures. The purity of all fractions was estimated using HPLC-PDA at 280 nm. Fraction 13 was found to contain 87% acrovestone, while fraction 71, 92% acrofolione A. Moreover, yellow crystals of acrovestone (**4**, 182.5 mg) precipitated from fraction 13. In order to isolate all prenylated acetophenone dimers present in the extract, FCPC fractions containing mixtures of acetophenones were purified further by semipreparative HPLC using an elution program of a 90 min linear gradient from 70% to 100% MeOH, then 10 min pure MeOH, 1 min back to initial conditions, and 9 min for re-equilibration (70% MeOH) with a flow rate of 5 mL/min (Figure H1, Supporting Information). Separation of a portion (80 mg, 10 mg per injection) of fraction 8 afforded acropyrone (**1**, 10.7 mg), and similarly a portion of fractions 17 and 18 (120 mg, 10 mg per injection) was subjected to semipreparative HPLC to obtain acropyranol A (**2**, 7.3 mg) and acrovestenol (**5**, 8.7 mg). Acropyranol B (**3**, 3.2 mg) and acrofolione B (**7**, 8.5 mg) were isolated from fraction 28 (100 mg, 10 mg per injection), while an additional quantity of acropyranol B (**3**, 2.8 mg) was isolated from fractions 33−39 (50 mg, 10 mg per injection). Finally, acronyline (**8**, 26.0 mg), a prenylated acetophenone monomer, was isolated as transparent crystals from fraction 69.

The plant residue after the first extraction with Et_2O was alkalinized with 10% NH_4OH and extracted successively with Et_2O, CH_2Cl_2, and MeOH (3 × 5 L, each solvent). The Et_2O extract (22.5 g) obtained after alkalinization was partitioned with 10% HCl (3 × 150 mL) until the negative reaction of the aqueous phase using Mayer's reagent and then alkalinized to pH 8−9 with 28% NH_4OH and extracted with CH_2Cl_2 (6 × 150 mL) using Mayer's reagent to control the process. An organic fraction (20.3 g) and an aqueous fraction (452 mg) rich in alkaloids were obtained after this procedure. The aqueous fraction was submitted to flash chromatography using CH_2Cl_2−MeOH (100:0 to 30:70) gradient solutions, which afforded four furoquinoline alkaloids, dictamnine (8.5 mg), pteleine (3.9 mg), evolitrine (26.6 mg), and kokusaginine (32.0 mg). Dictamnine and pteleine were isolated for the first time from this species.

*Acropyrone (**1**):* yellowish oil; $[\alpha]^{25}_{D}$ 0 (*c* 1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.13), 226 (4.15), 289 (4.12), and 333 (3.67, sh) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; APCI(+)-HRMS *m/z* 575.2610 [M + Na]$^+$ (3), 553.2791 [M + H]$^+$ (calcd for $C_{32}H_{41}O_8$, 553.2807) (13), 319.1905 (100), 303.1593 (16), 235.0966 (15).

*Acropyrranol A (**2**):* yellowish oil; $[\alpha]^{25}_{D}$ 0 (*c* 1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.47), 229 (4.42), 292 (4.37), 340 (3.98, sh) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; APCI(+)-HRMS *m/z* 571.2899 [M + H]$^+$ (calcd for $C_{32}H_{43}O_9$, 571.2913) (1), 319.1906 (100), 253.1073 (36).

*Acropyrranol B (**3**):* yellowish oil; $[\alpha]^{25}_{D}$ 0 (*c* 1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (4.38), 231 (4.36), 298 (4.29), 329 (4.06, sh) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; APCI(+)-HRMS *m/z* 593.2719 [M + Na]$^+$ (2), 571.2897 [M + H]$^+$ (calcd for $C_{32}H_{43}O_9$, 571.2913) (9), 319.1905 (100), 253.1072 (20).

Cell Lines and Culture. Human A2058 melanoma and DU145 prostate cancer cell lines were obtained from the American Type Culture Collection (ATCC). Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin. Normal human dermal fibroblast (NHDF) cells were purchased from Lonza. Cells were cultured in DMEM media containing 10% FBS.

Cell Viability Assays. Cell viability assays were performed as described previously.[19] Briefly, cells were seeded onto 96-well plates at a density of 5000 cells per well. After overnight incubation, cells were treated for 48 h with the isolated compounds **1**−**7** or DMSO as the vehicle control. MTS Reagent (CellTiter 96 AQueousOne Solution Cell Proliferation Assay; Promega) was added to each well according to the manufacturer's instructions. Absorbance was monitored at 490 nm using a microplate reader (Bio-Rad). Cell viability (%) was normalized to the vehicle control. Each experiment was performed in triplicate or quadruplicate. Sorafenib was used as positive control. IC_{50} values against DU145 and A2058 cells were 5.1 ± 0.7 and 3.8 ± 0.9 μM, respectively.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR and HRMS data of the isolated compounds **1**−**3**, **6**, and **7**, a HPLC chromatogram (280 nm) of the crude diethylether extract of *A. pedunculata*, representative CPC fractions as well as a table of inhibitory effects of compounds **1**−**7** against tested cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We express our thanks to T. L. Eng and D. M. Nor for the collection of plant material. This study was supported financially by "NatForce" (FP7-REGPOT-2007-1) under grant agreement number 206570.

DEDICATION

This paper is dedicated to the memory of our late colleague Professor François Tillequin.

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