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Acronyculatin P, A New Isoprenylated Acetophenone from the Stem Bark of *Acronychia pedunculata*

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Abstract – A new isoprenylated acetophenone, acronyculatin P (**1**) as well as two known compounds, 3',5'-diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**) and 3'-isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**) were isolated from the stem bark of *Acronychia pedunculata* (L.) Miq. The structures were determined by HRESIMS, 1D and 2D NMR. The inhibitory activity of the isoprenylated acetophenone derivatives against murine leukemia P-388 cells showed compound **1** moderate activity with IC_{50} 15.42 μM.
Keywords – Acronyculatin P, isoprenylated acetophenone, *Acronychia pedunculata*, P-388 cells

Introduction

Acronychia pedunculata is one species belongs to the Rutaceae family found in all of Indonesia. The stem bark have been used in traditional medicine for the treatment of fever, asthma, diarrhea, and rheumatism.[1] According to previous studies, the most common secondary metabolites isolated from *A. pedunculata* are alkaloids,[2] coumarins,[3] and isoprenylated acetophenone derivatives.[4,5] Isoprenylated acetophenone derivatives in the genus *Acronychia* indicate not their value as chemotaxonomic markers of the genus. Isoprenylated acetophenone derivatives were reported to possess cytotoxic,[4] anti-inflammatory,[6] and antioxidant[7] activities. In the present study, a phytochemical investigation is reported of the stem bark of *A. pedunculata* focused on the isolation and structural elucidation of a new isoprenylated acetophenone derivatives, acronyculatin P (**1**) along with two known compounds, 3',5'-diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**) and 3'-isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**). The cytotoxic activity of compounds **1** - **3** against murine leukemia P-388 cells from this plant are also reported.

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Experimental

General experimental procedures – Column chromatography and radial chromatography were carried out using silica gel 60 and silica gel 60 PF_{254} (Merck, Darmstadt, Germany). UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-VIS spectrophotometer (Kyoto, Japan). IR spectra were recorded in KBr on a One Perkin Elmer instrument (Waltham, MA, USA). NMR spectra were measured on a JEOL JNM-ECA 400 MHz FTNMR spectrophotometer (Tokyo, Japan) in $CDCl_3$ with TMS as the internal standard. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, $[M-H]^-$ negative ion mode (Santa Clara, CA, USA).

Plant materials – The dried and powdered of stem bark of *A. pedunculata* was collected in July 2017 from Gunung Salak, Bogor, West Java, Indonesia by Mr. Ismail Rachman. The plant material was identified at the Herbarium Bogoriense, Bogor. A voucher specimen (AP 60329) was deposited in Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and isolation – The stem bark of *A. pedunculata* (1.5 kg) was extracted with methanol at room temperature two times and then extracts were concentrated in vacuo. The methanol extract (350 g) was

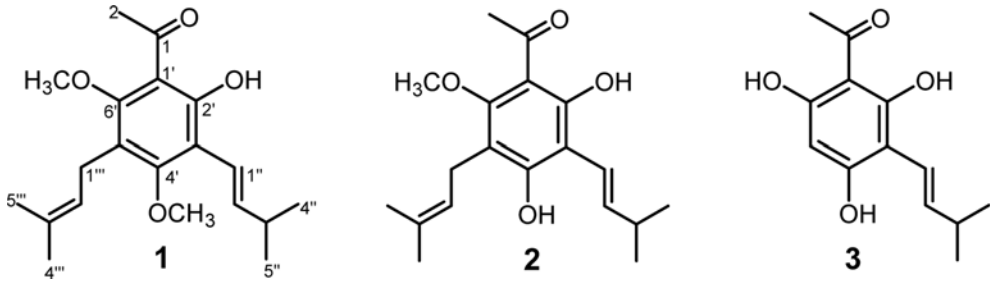

Fig. 1. Isoprenylated acetophenones **1** - **3** isolated from *A. pedunculata*.

suspended in H_2O and partitioned with *n*-hexane (11.6 g) and ethyl acetate (7.7 g). The *n*-hexane extract (11 g) was further fractionated by column chromatography on silica gel (200 g) eluted with *n*-hexane-ethyl acetate by increasing polarity (from 9:1, 4:1; 7:3, and 1:1) to give three major fractions A-C. Fraction A (0.5 g) was separated by planar radial chromatography eluted with *n*-hexane-$CHCl_3$ (from 9:1 to 4:1) to produce subfractions A_1-A_2. Subfraction A_2 was purified by planar radial chromatography using *n*-hexane-diisopropylether (from 9:1 to 4:1) to yield compound **1** (46 mg). Fraction B (5.3 g) was refractionated using column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 1:1) to produce three subfractions B_1-B_3. Subfraction B_2 was purified by planar radial chromatography using *n*-hexane-diisopropylether (from 9:1 to 4:1) to afford **2** (28 mg). The ethyl acetate extract (7.5 g) was fractionated over silica gel column chromatography eluted with *n*-hexane-ethyl acetate (from 9:1, 4:1; and 1:1) to give five major fractions D-H. Fraction E (0.75 g) was purified with *n*-hexane-ethyl acetate (from 4:1, 7:3; and 1:1) to afford **3** (24 mg).

Acronyculatin P (**1**) – Yellowish syrup. UV (MeOH) λ_{max} nm (log ε) : 252 (4.25), and 283 (4.23). IR (KBr) ν_{max} cm^{-1}: 3300, 1619, 1587 and 1184. ^1H and ^{13}C NMR see Table 1. HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{20}H_{28}O_4$ 331.1924, found 331.1909.

3',5'-Diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**) – Yellowish syrup. UV (MeOH) λ_{max} nm (log ε) : 224 (4.38), and 290 (4.21). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{19}H_{25}O_4$ 317.1536, found 317,1532.

3'-Isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**) – Pale yellowish solid, mp. 173 - 175 ºC. UV (MeOH) λ_{max} nm (log ε) : 236 (4.32), and 280 (4.20). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{13}H_{15}O_4$ 235.2569, found 235.2560.

Cytotoxic activity – The human tumor cell used in this work was P-388 cells (murine leukemia) and cultured in RPMI 1640 medium. The P-388 cells were seeded into each 96-well cell culture plate at a density of 3×10^4 cells/well and incubated at 37 ºC for 48 h against murine leukemia P-388 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay. Compounds **1-3** dissolved in DMSO by variations in concentration of 100; 30; 10; 3; 1; 0.3 and 0.1 μg/mL with triplicate treatment. The number of cells inhibited by each of compounds **1** - **3** were measured using microplate reader spectrometer at λ 540 nm. Artonin E was used as positive control and DMSO 1% was used as negative control.[8-11] The IC_{50} values of the compounds were calculated through extrapolation 50% absorption lines to various concentrations using regression analysis.

Result and Discussion

The structures of **1** - **3** were elucidated by UV, IR, HRESIMS, 1D and 2D NMR spectroscopy. To our knowledge, compound (**1**) is a new compound. The ^1H and ^{13}C NMR spectral data of compounds **2** - **3** are consistent with published data.[12-13]

Compound (**1**) was isolated as yellowish syrup. The HRESIMS of **1** exhibited a negative molecular ion peak $[M-H]^-$ at *m/z* 331.1924 indicating a molecular formula of $C_{20}H_{27}O_4$ implying seven degrees of unsaturation. The UV maximum absorption at λ_{max} 252 (4.25), 283 (4.23) nm and IR bands ($\nu_{max,}$ cm^{-1})at 3300, 1619, 1587 and 1184[4]. The ^1H NMR spectrum (Table 1) showed an acetyl signal at δ_H 2.71 (3H, s, H-2), a chelated hydroxyl group at δ_H 13.43 (1H, s, 2'-OH), and two methoxyl groups at δ_H 3.71 (6H, s, 4'/6'-OCH_3). In addition, compound **1** showed 3-methyl-1-butenyl proton signals at δ_H 6.40 (1H, d, *J* = 15.6 Hz, H-1"), 6.55 (1H, dd, *J* = 15.6; 7.1 Hz, H-2"), 2.48 (1H, dq, *J* = 15.6; 8.2 Hz, H-3"), 1.68 (3H, s, H-4"), 1.10 (6H, d, *J* = 6.8 Hz, H-4"/5"), and a 3-methyl-2-butenyl (isoprenyl) proton signals at δ_H 5.16 (1H, t, *J* = 6.6 Hz, H-2'"), 3.29 (2H, d, *J* = 6.6 Hz, H-1'"), 1.78 (3H, s, H-4'"), and 1.69 (3H, s, H-5'"). The placement of hydroxyl, methoxyl, 3-methyl-1-butenyl, and 3-methyl-2-butenyl groups of **1** were established by HMQC and HMBC spectra (Fig. 2). The ^{13}C NMR spectrum of **1**

Table 1. NMR Spectroscopic data (400 MHz in $CDCl_3$) for acronyculatin P (**1**)

No.C	δ_H (mult, *J* in Hz)	δ_C	HMBC
1	-	204.7	-
2	2.71 (*s*, 3H)	31.4	C-1
1'	-	111.9	-
2'	-	161.5	-
3'	-	116.4	-
4'	-	163.5	-
5'	-	120.4	-
6'	-	159.6	-
1"	6.40 (*d*, 15.6, 1H)	116.7	C-4', C-2", C-3"
2"	6.55 (*dd*, 7.1; 15.6, 1H)	143.1	C-1", C-3", C-4"/5"
3"	2.48 (*dq*, 8.2; 15.6, 1H)	32.9	C-1", C-2", C-4"/ C-5"
4"	1.10 (*d*, 6.8, 3H)	22.6	C-2", C-3", C-5"
5"	1.10 (*d*, 6.8, 3H)	22.6	C-2", C-3", C-4"
1"'	3.29 (*d*, 6.6, 2H)	23.2	C-4', C-5', C-6', C-2"', C-3"'
2"'	5.16 (*t*, 6.6, 1H)	123.8	C-4"', C-5"'
3"'	-	131.6	-
4"'	1.78 (*s*, 3H)	17.9	C-2"'. C-3"', C-5"'
5"'	1.69 (*s*, 3H)	25.8	C-2"'. C-3"', C-4"'
2'-OH	13.43 (*s*, 1H)	-	C-1', C-2', C-3'
4'-OCH_3	3.71 (*s*, 3H)	60.4	C-4'
6'-OCH_3	3.71 (*s*, 3H)	63.0	C-6'

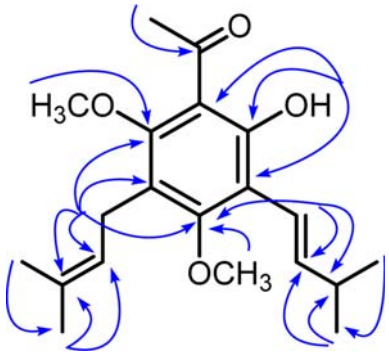

Fig. 2. Selected HMBC correlations for compound **1**.

revealed the signals for an acetyl group at δ_C 31.4 and δ_C 204.7 as well as three oxyaryl carbons at δ_C 163.5, δ_C 161.5, and δ_C 159.6 characteristic for a 2',4',6'- trioxygenated acetophenone derivatives.[5] The HMBC spectrum revealed a cross-peak correlation between δ_H 2.71 (H-2) and an carbonyl carbon at δ_C 204.7. The proton signal of a chelated hydroxyl group (δ_H 13.43, 2'-OH) correlated with three quaternary carbons [δ_C 111.9 (C-1'); 161.5 (C-2'); 116.9 (C-3')]. The proton signal of methoxyl group at δ_H 3.71 (4'/6'-OCH_3) correlated with two oxyaryl carbons [δ_C 163.5, and δ_C 159.6] showing the presence of two methoxyl groups at C-4' and C-6'. Furthermore, the proton signal of methylene of 3-methyl-2-butenyl chain at δ_H 3.29 has correlation with two oxyaryl carbons [δ_C 163.5 (C-4'), and δ_C 159.6 (C-6')], two quaternary carbons [δ_C 120.4 (C-5'), and δ_C 131.6 (C-3"')], and a methine carbon at δ_C 123.8 (C-2"') confirmed that the 3-methyl-2-butenyl chain is located at C-5'. The presence of long-range correlations between the proton signal of a vinylic of 3-methyl-1-butenyl chain at δ_H 6.40 (H-1") was correlated to a oxyaryl carbon at δ_C 163.5 (C-4'), and two methine carbons at δ_C 143.1 (C-2"), and 32.9 (C-3") reinforces the location of 3-methyl-1-butenyl chain at C-3'. Therefore, compound **1** was identified as (*E*)-1-(2'-hydroxy-4',6'-dimethoxy-3'-(3"-methylbut-1"-enyl)-5'-(3"'-methylbut-2"'-enyl)phenylethanone and given the trivial name acronyculatin P. Other HMBC correlations consistent with the structure **1** are shown in Table 1 and Fig. 2.

Compounds **1** - **3** were assessed for their cytotoxicity and results are shown in Table 2. The IC_{50} values were 15.42, 27.26, and 80.59 μM, respectively (artonin E as a positive control, IC_{50} 3.05 μM). Compound **1** was more active than others, But, all the compounds were less active when compared with the positive control.

Table 2. Cytotoxic activity of compounds **1 - 3** against murine leukemia P-388 cells

Compounds	IC_{50} (μM)
Acronyculatin P (**1**)	15.42 ± 0.51
3',5'-Diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**)	27.26 ± 1.23
3'-Isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**)	80.59 ± 1.67

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