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Potential anti-allergic *ent*-kaurene diterpenes from the bark of *Suregada multiflora*

Sarot Cheenpracha [a], Orapun Yodsaoue [a], Chatchanok Karalai [a,*], Chanita Ponglimanont [a], Sanan Subhadhirasakul [b], Supinya Tewtrakul [b], Akkharawit Kanjana-opas [c]

[a] *Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[b] *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[c] *Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*

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Abstract

Two *ent*-kaurene diterpenes, *ent*-16-kaurene-3β,15β,18-triol (**1**) and *ent*-3-oxo-16-kaurene-15β,18-diol (**2**), were isolated from a dichloromethane extract of the bark of *Suregada multiflora* along with five known diterpenes:*ent*-16-kaurene-3β,15β-diol (**3**), abbeokutone (**4**), helioscopinolide A (**5**), helioscopinolide C (**6**) and helioscopinolide I (**7**). Their structures were elucidated on the basis of spectroscopic analysis. Compounds **1**–**7** possessed appreciable anti-allergic activities in RBL-2H3 cells model with IC_{50} values ranging from 22.5 to 42.2 μM.

Keywords: *Suregada multiflora*; *ent*-Kaurene type diterpenes; Anti-allergic activity

1. Introduction

Suregada multiflora A. Juss. (syn. *Gelonium multiflorum*), known locally as "Yaipluak" in Thai, is a plant belonging to the Euphorbiaceae and distributed in the tropical and subtropical areas of Asia and Africa. *S. multiflora* is the only species found in Thailand. This plant has been reported to contain anti-human immunodeficiency virus type 1 (HIV-1) protein, GAP31, and also exhibit the inhibitory effect on the infection and replication of herpes simplex virus (HSV) (Bourinbaiar and Lee-Huang, 1996). The crude (CH_2Cl_2–MeOH, 1:1) extract of this plant exhibited selective cytotoxic activity in different human tumor cell lines (Jahan et al., 2002). Thai traditional medicine uses the bark to treat inflammation and skin diseases (Wutthithamavet, 1997).

Hypersensitivity type I or allergy is caused by certain types of antigens such as dust, mites, medicines, foods, pollens, spores, and cosmetics. This class of antigens induces the production of antigen-specific IgE antibodies that bind to receptors on mast cells or basophiles; and finally cause mast cells or basophiles to degranulate and secrete mediators that induce vasodilation, mucous secretion and bronchoconstriction. β-Hexosaminidase is the enzyme that is stored in the secretory granules of mast cells and basophiles, and is released along with histamine when mast cells and basophiles are activated. Thus, this enzyme is used as the marker of mast cell or basophile degranulation (Cheong et al., 1998). Previous chemical investigations on different parts of *S. multiflora* have resulted in the isolation of several diterpenoids (Choudhary et al., 2004; Das et al., 1994; Jahan et al., 2002, 2004) and flavonoids (Das and Chakravarty, 1993; Parveen and Khan, 1987). We report herein the isolation of two new *ent*-kaurene diterpenoids (**1**–**2**) together with five known diterpenoids from the active

* Corresponding author. Tel.: +66 7428 8444; fax: +66 7421 2918.
E-mail addresses: chatchanok.k@psu.ac.th, chatchanok_k@yahoo.com (C. Karalai).

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dichloromethane extract of the bark of *S. multiflora*; *ent*-16-kaurene-3β,15β-diol (**3**) (Das et al., 1994), abbeokutone (**4**) (Agrawal et al., 1995), helioscopinolide A (**5**) (Borghi et al., 1991), helioscopinolide C (**6**)(Borghi et al., 1991) and helioscopinolide I (**7**) (Crespi-Perellino et al., 1996). In addition, their anti-allergic activity is also discussed.

2. Results and discussion

Compound **1** was isolated as a white solid. It showed the $[M]^+$ at *m/z* 320.2351 ($C_{20}H_{32}O_3$) in the HREIMS spectrum. The presence of hydroxyl (3337 cm^{-1}) functionality was evident from IR absorption. The ^{13}C NMR and DEPT spectral data (Table 1) exhibited 20 carbons, attributable to two methyl, nine methylene, five methine and four quaternary carbons indicating a diterpenoid. Two low-field signals at δ 108.3 and 159.1 represented two carbons of an exocyclic double bond and the signals at δ 82.6, 74.9 and 69.5 indicated the presence of three oxygenated carbons in the molecule. The ^1H NMR spectral data (Table 1) displayed the presence of two singlets at δ 1.02 (3H, *s*, Me-20) and 0.80 (3H, *s*, Me-19), a set of methylene protons at δ 5.19, 5.09 (each 1H, *br s*, 2H-17), two oxymethine protons at δ 3.59 (1H, *dd*, $J = 10.8$, 6.0 Hz, H-3) and 3.78 (1H, *s*, H-15), and a methine proton at δ 2.73 (1H, *br s*, H-13). These data indicated that compounds **1** and **3** (Das et al., 1994) were closely related, except for the replacement of the methyl proton signal at δ 1.00 in **3** with oxymethylene proton signals at δ 3.58 and 3.33 (each 1H, *d*, $J = 10.8$ Hz, 2H-18) in **1**. The location of the oxymethylene protons was established as follows. An oxymethine proton at δ 3.59 (H-3) showed HMBC correlations with the carbons at δ 69.5 (C-18), 48.7 (C-5), 26.1 (C-2) and 11.5 (C-19), a methine proton at δ 0.90 (H-5) with the carbons at δ 69.5 (C-18), 54.2 (C-9), 41.7 (C-4), 38.9 (C-10), 17.9 (C-20) and 11.5 (C-19), and the methyl protons at δ 0.80 (Me-19) with the carbons at δ 74.9 (C-3), 69.5 (C-18), 48.7 (C-5) and 41.7 (C-4), confirming the attachment of oxymethylene protons at C-18. Compound **1** was assumed to belong to the *ent*-kaurene series and its relative stereochemistry was established by a NOESY experiments and on the basis of coupling constants. The large *J* value of proton H-3 ($J = 10.8$ Hz) indicated an axial orientation (β-face). Me-20 showed NOESY cross peaks with Me-19 and H-14α. On the other hand, the cross-peaks between H-3/H-5, H-5/H-9 and H-9/H-15 suggested the β-orientation

Table 1
^1H and ^{13}C NMR spectral data of compounds **1**–**3** in $CDCl_3$ (300 and 75 MHz)

Position	**1**		**2**		**3**	
	δ_C	δ_H (mult., *J*, Hz)[a]	δ_C	δ_H (mult., *J*, Hz)[a]	δ_C	δ_H (mult., *J*, Hz)[a]
1	38.4	1.85 (*m*)	38.6	2.12 (*dd*, 13.2, 2.7)	38.7	1.87 (*m*)
				2.09 (*dd*, 13.2, 2.7)		0.91 (*m*)
2	26.1	1.60 (*m*)	35.4	2.61 (*ddd*, 16.8, 12.3, 7.2)	27.1	1.69 (*m*)
				2,35 (*ddd*, 16.8, 6.6, 2.7)		1.63 (m)
3	74.9	3.59 (*dd*, 10.8, 6.0)	219.0		79.0	3.21 (*dd*, 10.8, 5.4)
4	41.7		52.2		38.8	
5	48.7	0.90 (*m*)	48.6	1.74 (*m*)	55.0	0.76 (*dd*, 8.1, 2.7)
6	19.0	1.63 (*m*)	20.2	1.67 (*m*)	19.2	1.72 (*m*)
		1.42 (*m*)		1.59 (*m*)		1.63 (*m*)
7	34.7	1.63 (*m*)	34.2	1.78 (*br, dd*, 9.6, 3.3)	35.2	1.75 (*m*)
		1.39 (*m*)		1.56 (*m*)		1.47 (*m*)
8	47.3		52.7		47.5	
9	54.2	1.00 (*m*)	54.2	1.16 (*br d*, 6.6)	54.1	1.01 (*m*)
10	38.9		38.5		39.2	
11	18.0	1.54 (*m*)	18.5	1.51 (*m*)	18.1	1.43 (*m*)
				1.48 (*m*)		1.29 (*m*)
12	32.6	1.48 (*m*)	32.5	1.74 (*m*)	32.7	1.67 (*m*)
						1.54 (*m*)
13	42.2	2.73 (*br s*)	42.1	2.79 (*br s*)	42.3	2.75 (*br s*)
14	36.1	1.85 (*m*)	36.2	1.92 (*br d*, 12.0)	36.2	1.90 (*m*)
		1.35 (*m*)		1.46 (*m*)		
15	82.6	3.78 (*s*)	82.6	3.84 (*s*)	82.9	3.80 (*s*)
16	159.1		159.8		160.3	
17	108.3	5.19 (*br s*)	108.7	5.24 (*br s*)	108.3	5.21 (*br s*)
		5.09 (*br s*)		5.11 (*br s*)		5.08 (*br t*, 0.6)
18	69.5	3.58 (*d*, 10.8)	67.3	3.64 (*d*, 11.1)	28.4	1.00 (*s*)
		3.33 (*d*, 10.8)		3.43 (*d*, 11.1)		
19	11.5	0.80 (*s*)	16.8	1.03 (*s*)	15.5	0.79 (*s*)
20	17.9	1.02 (*s*)	17.5	1.20 (*s*)	17.6	1.03 (*s*)

[a] Assignments were made using HMQC and HMBC data.

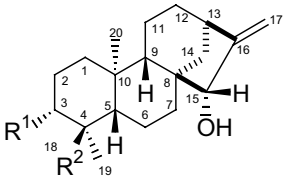
Table 2
Anti-allergic activity against antigen-induced β-hexosaminidase release of compounds **1**–**7** from *S. multiflora*

Compound	IC_{50} (μM)	Enzyme inhibition at 100 μM (%)
ent-16-Kaurene-3β,15β,18-triol (**1**)	22.5	14.3
ent-3-Oxo-16-kaurene-15β,18-diol (**2**)	22.9	17.9
ent-16-Kaurene-3β,15β-diol (**3**)	28.7	21.0
Abbeokutone (**4**)	42.1	16.1
Helioscopinolide A (**5**)	26.5	19.5
Helioscopinolide C (**6**)	37.0	18.6
Helioscopinolide I (**7**)	29.3	21.7
Quercetin	4.5	16.8
Ketotifen fumarate	47.5	15.8

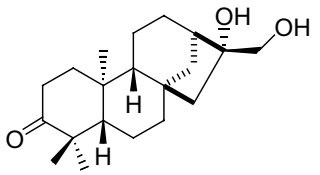
of H-3, H-5, H-9 and H-15. The appearance of an oxymethine proton H-15 as a singlet also supported its β-orientation (Jia et al., 1994). The relative stereochemistry at the important chiral centers should be a reasonable assumption since several other *ent*-kaurenes have been reported from the plant (Jahan et al., 2004; Das et al., 1994). Therefore, the structure of compound **1** was elucidated as *ent*-16-kaurene-3β,15β,18-triol.

Compound **2**, $C_{20}H_{30}O_3$ ($[M]^+$ *m/z* 318.2195), was obtained as a colorless, viscous oil. The IR (3407 and 1694 cm^{-1}) absorption bands were characteristic of hydroxyl and carbonyl groups, respectively. The ^{13}C NMR spectral data (Table 1) showed a total of 20 carbons with one carbonyl carbon at δ 219.0. The ^1H and ^{13}C NMR spectra of **2** resembled those of **1** except that the hydroxyl at C-3 in **1** was replaced by a keto group in **2** as the disappearance of an oxymethine proton signal (H-3) of **1** at δ 3.59. This finding was supported by the HMBC spectrum of **2**, in which the methyl protons at δ 1.03 (Me-19) correlated with the carbons at δ 219.0 (C-3), 67.3 (C-18), 52.2 (C-4) and 48.6 (C-5) and oxymethylene protons at δ 3.64 and 3.43 (2H-18) with the carbons at δ 219.0 (C-3), 48.6 (C-5) and 16.8 (C-19). Thus, compound **2** was determined as *ent*-3-oxo-16-kaurene-15β,18-diol.

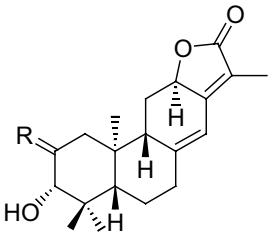
As shown in Table 2, *ent*-16-kaurene-3β,15β,18-triol (**1**) exhibited appreciable anti-allergic activity against antigen-induced β-hexosaminidase release with an IC_{50} value of 22.5 μM, followed by *ent*-3-oxo-16-kaurene-15β,18-diol (**2**, $IC_{50} = 22.9$ μM), helioscopinolide A (**5**, $IC_{50} = 26.5$ μM), *ent*-16-kaurene-3β,15β-diol (**3**, $IC_{50} = 28.7$ μM), helioscopinolide I (**7**, $IC_{50} = 29.3$ μM), helioscopinolide C (**6**, $IC_{50} = 37.0$ μM) and abbeokutone (**4**, $IC_{50} = 42.1$ μM). All these compounds possessed stronger anti-allergic activity than ketotifen fumarate, a clinically used drug ($IC_{50} = 47.5$ μM), but lower activity than quercetin ($IC_{50} = 4.5$ μM), a positive reference compound. Compounds **1**–**7** were also tested on the enzyme activity of β-hexosaminidase and showed weak inhibition at 100 μM, whose results indicated the inhibition of the antigen-induced degranulation but not the activity of β-hexosaminidase.



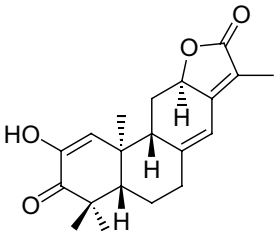
1 : R^1 = OH, R^2 = CH_2OH
2 : R^1 = =O, R^2 = CH_2OH
3 : R^1 = OH, R^2 = CH_3



4



5 : R = H_2
6 : R = O



7

In conclusion, the anti-allergic activity of compounds **1**–**7** isolated from the bark of *S. multiflora* seem to support the traditional use of this plant for skin disease and inflammation treatments. The phytochemical study has also led to the isolation of three *ent*-kaurenes (**1**–**3**), an *ent*-kaurane (**4**) and tetracyclic diterpene lactones (**5**–**7**). These compounds will provide the platform for structure-based design of an additional class of inhibitors for anti-allergic therapy.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Fisher-John melting point apparatus. The specific rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with a SPECORD S 100 (Analytikjena). The IR spectra were measured with a Perkin–Elmer FTS FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded using a 300 MHz Bruker FTNMR Ultra Shield™ spectrometer. Chemical shifts are recorded in part per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254}(Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

3.2. Plant material

Bark of *S. multiflora* was collected from Songkhla province, Thailand in November 2004. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology,

Faculty of Science, Prince of Songkla University and a specimen (No. SC04) deposited at Prince of Songkla University Herbarium.

3.3. Extraction and isolation

Ground-dried bark (5.9 kg) of *S. multiflora* was extracted with CH_2Cl_2 (2×7.5 L, for 5 days) at room temp. The crude extract was evaporated under reduced pressure to afford a brownish CH_2Cl_2 extract (27.3 g). The CH_2Cl_2 extract was further purified by QCC using hexane as eluent and increasing polarity with acetone and MeOH to give seven fractions (F1–F7). Fraction F2 (702.9 mg) was subjected to CC with EtOAc–hexane (1:3, v/v) and followed by prep TLC with MeOH–CH_2Cl_2 (1:49, v/v) to give **2** (3.3 mg) and **4** (7.2 mg). Fraction F4 (1.8 g) was purified by CC with acetone–CH_2Cl_2 (1:49, v/v) to afford four subfractions. Subfraction F4b (67.4 mg) was separated by CC with EtOAc–hexane (3:7, v/v) to afford **5** (12.6 mg). Subfraction F4d (370.2 mg) was purified by CC with EtOAc–hexane (3:7, v/v) and followed by prep TLC with EtOAc–hexane (3:7, v/v) to give **3** (6.2 mg), **6** (9.1 mg) and **7** (8.3 mg). Fraction F6 (1.2 g) was subjected to CC with MeOH–CH_2Cl_2 (1:19, v/v) to afford three subfractions (F6a–F6c). Subfraction F6c (327.7 mg) was further purified by CC with acetone–CH_2Cl_2 (1:9, v/v) to give **1** (22.5 mg).

3.3.1. *ent*-16-Kaurene-3β,15β,18-triol (**1**)

White solid, m.p. 205–206 °C; $[\alpha]_D^{27}$ −2.5° (*c* 0.04, MeOH); UV λ_{max} (MeOH) (log ε): 206 (3.30) nm; IR (KBr) ν_{max}: 3337 (OH) cm^{-1}; EIMS: *m*/*z* 320 (23)(M^+), 302 (63), 287 (85), 271 (100); HREIMS: *m*/*z* $[M]^+$ 320.2311 (calcd. for $C_{20}H_{32}O_3$, 320.2351); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see Table 1.

3.3.2. *ent*-3-Oxo-16-kaurene-15β,18-diol (**2**)

Colorless viscous oil; $[\alpha]_D^{27}$ +35.7° (*c* 0.06, MeOH); UV λ_{max} (MeOH) (log ε): 205 (2.87) nm; IR (KBr) ν_{max}: 3407 (OH), 1694 (>C=O) cm^{-1}; EIMS: *m*/*z* 318 (23)(M^+), 288 (100), 273 (25), 159 (33), 84 (40);HREIMS: *m*/*z* $[M]^+$ 318.2191 (calcd. for $C_{20}H_{30}O_3$, 318.2195); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see Table 1.

3.4. Bioassays

3.4.1. Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 were evaluated by the following method (Matsuda et al., 2002).

3.4.2. β-Hexosaminidase inhibitory activity

In order to clarify that the anti-allergic effects of samples were due to the inhibition on β-hexosaminidase release and not β-hexosaminidase activity the following assay was carried out. The cell suspension (5×10^7 cells) in 6 ml of PBS was sonicated. The solution was then centrifuged; and the supernatant diluted with Siraganian buffer and adjusted to equalize the enzyme activity of the degranulation tested above. The enzyme solution (45 μl) and test sample solution (5 μl) were transferred into a 96-well microplate and incubated with 50 μl of the substrate solution at 37 °C for 1 h. The reaction was stopped by adding 200 μl of the stop solution. The absorbance was measured using a microplate reader at 405 nm and the results were expressed as mean ± SEM of four determinations. The IC_{50} values were calculated using the Microsoft Excel program. The statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

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