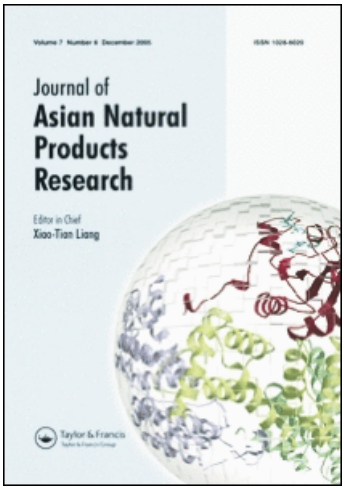

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New cytotoxic steroids from the fruits of *Syzygium siamense*

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NOTE

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A new sterol, stigmast-5-ene-3β,17α-diol (**1**), together with six known compounds, stigmast-5-ene-3β-yl formate (**2**), stigmast-5-ene-3β,7α-diol (**3**), stigmast-5-ene-7α-methoxy-3β-ol (**4**), stigmast-5-ene-3-one (**5**), 3β-sitostanol (**6**), and 3β-sitosterol (**7**), was isolated from the fruits of *Syzygium siamense*, of which compound **2** is reported for the first time from a natural source. Their structures were elucidated by spectroscopic methods. The isolated compounds (**1**–**7**) were evaluated for their cytotoxic activities against human oral epidermoid carcinoma cancer (KB), human breast cancer (BC), and human small cell lung cancer (NCI-H187) cell lines.

Keywords: *Syzygium siamense*; Myrtaceae; steroidal; cytotoxicity

1. Introduction

Syzygium siamense belongs to the family Myrtaceae, which is distributed in several Southeast Asian countries. This plant has been used by the local Thai people in folk medicine for its diuretic, odontalgic, stomachic, and stimulant properties [1]. Volatile oils from the species of *Syzygium* exhibit antibacterial activity [2,3]. Previous work on this genus reported their anti-inflammatory, antihypertensive, anticonvulsant, and antimicrobial activities [4–8], but until today, no phytochemical studies have been carried out to identify the active metabolites. As a part of our search for bioactive metabolites from Thai medicinal plants [9–11], we investigated the hexane and ethyl acetate soluble extracts from the fruits of *S. siamense* and it was found to exhibit significant cytotoxic activity when evaluated against a panel of human cell lines.

Fractionation of the hexane and ethyl acetate extracts from the fruits of *S. siamense* led to the isolation of a new naturally occurring stigmast-5-ene-3β,17α-diol (**1**), along with six known sterols, stigmast-5-ene-3β-yl formate (**2**), stigmast-5-ene-3β,7α-diol (**3**), stigmast-5-ene-7α-methoxy-3β-ol (**4**), stigmast-5-ene-3-one (**5**), 3β-sitostanol (**6**), and 3β-sitosterol (**7**) (Figure 1). The structures of the known compounds were elucidated by comparison of their physical and spectral data with the literature values [12–15]. The obtained sterol derivatives, **1**–**7**, were evaluated biologically against human cancer cell lines.

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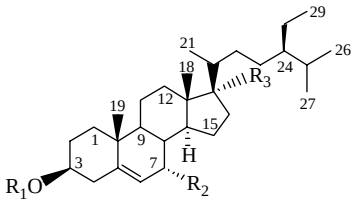
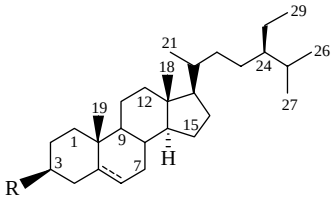


	R_1	R_2	R_3
1	H	H	OH
2	CHO	H	H
3	H	OH	H
4	H	OMe	H



5 R = O; C5–C6 unsaturated
6 R = OH; C5–C6 saturated
7 R = OH; C5–C6 unsaturated

Figure 1. Structures of compounds **1**–**7** from *S. siamense*.

2. Results and discussion

Sterol (**1**) was isolated as a colorless gum, and the molecular formula $C_{29}H_{50}O_2$ was established by HR-FAB-MS at *m/z* 430.3809 $[M]^+$. Its IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and trisubstituted olefinic groups (845 and 1670 cm^{-1}). The steroid nature of this compound was deduced from a combination of ^{13}C NMR and DEPT-135 spectra, which showed that the compound had 29 carbon atoms, including 4 quaternary carbons, 8 methines, 11 methylenes, and 6 methyl groups. One oxygenated methine carbon resonated at δ 71.8 (C-3), one oxygenated quaternary carbon at δ 82.5 (C-17), and two trisubstituted double bond carbon atoms C-5 and C-6 at δ 140.6 and 121.7, respectively. The ^1H NMR spectrum (Table 1) indicated the presence of characteristic steroidal methyl signals; two methyl singlets resonated at δ 0.65 (Me-18) and 0.98 (Me-19), and four methyl doublets at δ 0.79 ($J = 6.5$ Hz, Me-26), 0.81 ($J = 6.5$ Hz, Me-29), 0.85 ($J = 6.9$ Hz, Me-27), and 0.90 ($J = 6.9$ Hz, Me-21). The characteristic H-6 of steroid 5,6-enes was noticed at δ 5.33 as a doublet ($J = 5.5$ Hz). The presence of a 3β oxygenation was indicated by a broad methine multiplet at δ 3.51. In the COSY spectrum, the broad multiplet signal for H-3 showed coupling to H-4 protons resonating at δ 2.31 and 2.26, which were in turn coupled to H-2 appearing at δ 1.86. The results from the HMBC experiment indicated long-range correlations from H-3 to C-1 and C-4, from H-6 to C-5, C-7, and C-10. In addition to C-5 at δ 140.6, C-10 at δ 42.2, and C-13 at δ 42.1, compound **1** possessed a fourth quaternary carbon at δ 82.5, suggesting an oxygen-bearing carbon. To be in accordance with the HMBC correlations between this carbon (δ 82.5) and H-16, H-18, and H-21, the hydroxyl group had to be located on C-17. Consequently, the chemical shift of C-18 was shifted downfield from δ 11.8 to 14.2, when compared to the sitosterol [16,17]. The NOESY correlations between Me-18β, H-8, and H-20 suggested the β-orientation for C-20 and, consequently, the α-orientation for the hydroxyl group, features normally exhibited by a steroid skeleton. Assignments of proton and carbon signals for the side chain of **1** were strongly supported by the literature data [18–20]. On the basis of the above evidence, the structure of **1** was characterized as stigmast-5-ene-3β,17α-diol.

Sterol (**2**) was determined as stigmast-5-ene-3β-yl formate, and its structure was established by mass spectrometry, 1D and 2D NMR techniques and by comparison with those available in the literature [12]. This compound is reported for the first time from a natural source.

The isolated compounds **1**–**7** were evaluated for their cytotoxic activities against human oral epidermoid carcinoma cancer (KB), human breast cancer (BC), and human small cell lung cancer

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **1**[a] and **2**[a].

	1		**2**	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	37.2	1.13 (m)	37.6	1.11 (m)
		1.84 (m)		1.86 (m)
2	31.8	1.56 (m)	27.8	1.53 (m)
		1.86 (m)		1.94 (m)
3	71.8	3.51 (m)	73.9	4.72 (m)
4	39.7	2.26 (m)	39.3	2.24 (m)
		2.31 (m)		2.31 (m)
5	140.6		139.2	
6	121.7	5.33 (d, 5.5)	122.9	5.37 (d, 5.0)
7	31.5	1.53 (m)	31.5	1.54 (m)
		2.01 (t, 3.5)		2.02 (t, 3.5)
8	31.8	1.65 (m)	31.4	1.67 (m)
9	50.0	1.26 (m)	49.6	1.21 (m)
10	42.2		39.6	
11	21.0	1.51 (m)	21.0	1.51 (m)
		1.62 (m)		1.61 (m)
12	36.4	1.67 (m)	36.5	1.65 (m)
13	42.1		42.2	
14	56.7	1.66 (m)	56.6	1.66 (m)
15	24.2	1.15 (m)	24.2	1.18 (m)
		1.75 (m)		1.76 (m)
16	26.0	1.46 (m)	26.0	1.40 (m)
		2.23 (m)		2.22 (m)
17	82.5		55.9	
18	14.2	0.65 (s)	11.8	0.65 (s)
19	19.3	0.98 (s)	19.2	1.01 (s)
20	36.1	1.59 (m)	36.1	1.58 (m)
21	18.7	0.90 (d, 6.9)	18.7	0.91 (d, 6.5)
22	33.9	1.02 (m)	33.8	1.04 (m)
		2.02 (m)		1.99 (m)
23	28.2	1.04 (m)	27.7	1.06 (m)
		1.35 (m)		1.39 (m)
24	45.7	0.95 (m)	45.7	0.96 (m)
25	29.0	1.68 (m)	29.0	1.67 (m)
26	19.0	0.79 (d, 6.5)	19.0	0.79 (d, 7.0)
27	19.8	0.85 (d, 6.9)	19.8	0.85 (d, 7.0)
28	23.0	1.16 (m)	23.0	1.15 (m)
		1.32 (m)		1.30 (m)
29	11.9	0.81 (d, 6.5)	11.9	0.82 (d, 7.0)
OCHO	–	–	160.6	8.01 (s)

Notes: [a]TMS was used as an internal standard, and chemical shifts (δ) are presented in parts per million. *J* values are given in Hz in parentheses.

(NCI-H187) cell lines as shown in Table 2. The sterol derivatives **1**, **3**, and **4** showed moderate inhibitory effect against KB, BC, and NCI-H187 cells with IC_{50} values from 6.63 to 14.86 μg/ml. Compound **2** exhibited moderate cytotoxicity against BC and NCI-H187 cell lines with IC_{50} values of 17.63 and 10.67 μg/ml, respectively, whereas compounds **6** and **7** were found to be exhibiting selective cytotoxic activity against the NCI-H187 cell line. It should be noted that the hydroxyl group

Table 2. Cytotoxic activities of compounds **1**–**7**.

Sample	Cell lines[a]		
	KB	BC	NCI-H187
1	10.67	14.86	6.63
2	22.89	17.63	10.67
3	13.89	10.17	9.45
4	12.25	10.76	10.33
5	>50	>50	>50
6	>50	>50	26.45
7	>50	>50	20.43

Note: [a]Results are expressed as IC_{50} values (μg/ml); activity: <5, strong; 5–20, moderate; 20–50, weak; >50, inactive.

at C-7/C-17 is important for cancer activity in a steroid skeleton, and it seemed to have increased the cytotoxicity.

3. Experimental

3.1 General experimental procedures

Specific rotations were determined with an Autopol II automatic polarimeter. UV spectra were measured with a UV-160A spectrophotometer (Shimadzu, Kyoto, Japan), and IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a 500 MHz Varian Unity INOVA spectrometer. Chemical shifts are recorded in parts per million (δ) in $CDCl_3$. Mass spectra (EI or FAB) were recorded on a Finnigan-MAT 95 XL spectrometer. Column chromatography was carried out on silica gel 60 GF_{254} (Merck, Darmstadt, Germany).

3.2 Plant material

The fruits of *S. siamense* were collected in Khian Sa, Suratthani, Thailand, in March 2008. A voucher specimen (No. WU-0198) has been deposited in the herbarium of Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

3.3 Extraction and isolation

The dried fruits of *S. siamense* (2.1 kg) were extracted with hexane and EtOAc at room temperature. The hexane extract (4.5 g) was subjected to column chromatography over silica gel and eluted with a gradient of hexane–EtOAc to afford four fractions (H1–H4). Fraction H2 (0.9 g) was purified by column chromatography using EtOAc–hexane (1:4) to give compound **1** (6.7 mg). Fraction H3 (0.4 g) was subjected to silica gel column chromatography eluted with EtOAc–hexane (1:4) to yield compound **6** (14.2 mg). The EtOAc extract (10.3 g) was chromatographed on a silica gel column and eluted with a gradient system of hexane–EtOAc to afford six fractions (E1–E6). Compounds **2** (11.3 mg) and **5** (8.7 mg) were derived from fraction E3 (1.8 g) by column chromatography using EtOAc–hexane (1:4) as the eluent. Fraction E2 (1.1 g) eluted with EtOAc–hexane (1:9) yielded compound **3** (9.2 mg) after purification by column chromatography. Fraction E5 (0.8 g) was purified by column chromatography using EtOAc–hexane (1:4) to give compound **4** (7.7 mg). Fraction E4 (1.2 g) was separated on a silica gel column eluting with EtOAc–hexane (1:3) to afford compound **7** (13.5 mg).

3.3.1 Compound (*1*)

Colorless gum; $[\alpha]_D^{28}$ −35.2 ($c = 0.010$, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3450, 1670, 845 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS *m/z*: 430.3809 $[M]^+$ (calcd for $C_{29}H_{50}O_2$, 430.3811).

3.3.2 Compound (*2*)

Colorless gum; $[\alpha]_D^{28}$ −23.7 ($c = 0.040$, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 1734, 1643, 840 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS *m/z*: 442.3818 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 442.3811).

3.4 Cytotoxicity assay

The cytotoxicity assay employed the colorimetric method [21]. Ellipticine, the reference substance, exhibited activity toward KB, BC, and NCI-H187 cell lines, with the IC_{50} range of 0.3–0.6 μg/ml (Table 2).

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