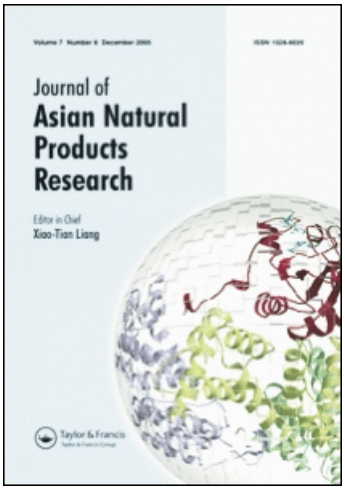

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new cytotoxic acetogenins from *Annona squamosa*

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To cite this Article Yang, Hai-Jun , Li, Xiang , Zhang, Ning , Chen, Jian-Wei and Wang, Ming-Yan(2009) 'Two new cytotoxic acetogenins from *Annona squamosa*', Journal of Asian Natural Products Research, 11: 3, 250 — 256
To link to this Article: DOI: 10.1080/10286020802682916
URL: http://dx.doi.org/10.1080/10286020802682916

Journal of Asian Natural Products Research
Vol. 11, No. 3, March 2009, 250–256

Two new cytotoxic acetogenins from *Annona squamosa*

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(*Received 25 September 2008; final version received 4 December 2008*)

Two new annonaceous acetogenins named as squamostanin-A and squamostanin-B were isolated from 95% EtOH extract of the seeds of *Annona squamosa*. Their structures were determined by spectroscopic method, and their cytotoxicities were evaluated using MTT method.

Keywords: *Annona squamosa*; annonaceous acetogenins; cytotoxic activity

1. Introduction

Annonaceous acetogenins (ACGs) constitute a series of natural products isolated exclusively from *Annonaceae* plants [1–5], which comprise of some 130 genera and include over 2300 species and are widely distributed in tropical and subtropical regions. The main chemical ingredients of *Annonaceae* plants are ACGs and alkaloids. Now, more than 500 ACGs have been isolated from *Annona* genus plant. ACGs exhibited a broad range of biological properties such as cytotoxic, antiparasitic, pesticidal, and immunosuppressive activities [6,7]. Especially, the ACGs have distinctive antitumor effects through the depletion of ATP levels via inhibiting complex I of mitochondria and inhibiting the NADH oxidase of plasma membranes of tumor cells [8,9]. In our investigation, two new ACGs were isolated from the seeds of *Annona squamosa*. In this paper, we report the isolation, structure elucidation, and cytotoxicities of two new ACGs (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{37}H_{68}O_8$ by the ion peak at m/z 639.4698 $[M + H]^+$ in the HR-EI-MS. The IR absorption bands at 3320, 1720, and 1524 cm^{-1} showed the presence of hydroxyl, carbonyl, and double bond. In the ^1H NMR spectrum of **1**, the signals at δ 7.20 (1H, d, H-35), 5.07 (1H, dq, H-36), 2.26 (2H, t, H-3), and 1.38 (3H, d, H-37), together with the carbon signals at δ 174.5 (C-1), 152.2 (C-35), 134.5 (C-2), 79.5 (C-36), 25.4 (C-3), and 19.2 (C-37) are characteristic spectral data for the methyl α,β-unsaturated γ-lactone moiety, commonly found in the ACGs [10], which was also supported by the positive Kedde reaction. The presence of a non-adjacent bis-THF ring system with four flanking hydroxyls was indicated by the ^1H NMR signals at δ 3.83–3.93 (4H, m, H-11, 14, 19, 22), 3.31–3.40 (3H, m, H-15, 18, 23), and 3.40 (1H, m, H-27), and the ^{13}C NMR signals at δ 80.9 (C-11), 84.0 (C-14), 83.8 (C-19), 83.9 (C-22), 75.5 (C-15), 75.3 (C-18), 75.1 (C-23), and 74.4 (C-27). In the high-field region of the ^1H and ^{13}C NMR spectra, the proton signals at δ 1.23–1.76 (m) and the carbon signals at δ 14.4–32.6 showed the presence of long fatty chain. Furthermore, the EI-MS fragment ions at 539, 521, 503, 485, 467, 449, 431, 413, 367,

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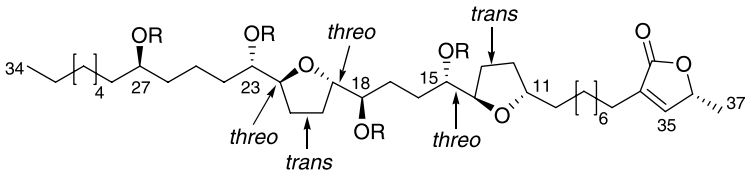
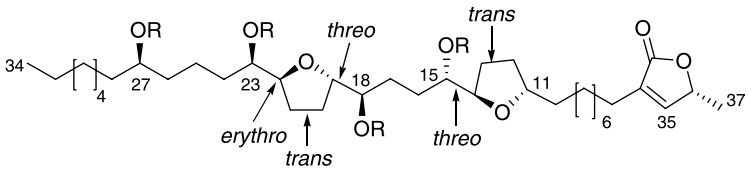
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802682916
http://www.informaworld.com



1 R = H **1a** R = Ac **1b** R = TMSi



2 R = H **2a** R = Ac **2b** R = TMSi

Figure 1. Structures of compounds **1** and **2**.

and 349 showed the clearance process of compound **1** (Figure 2), the four successive losses of H_2O (*m*/*z* 18) in the EI-MS of **1** showed the existence of four OH moieties, and the positions of the OH groups in **1** were assigned at C-15, C-18, C-23, and C-27 by careful analysis of fragment ions in the EI-MS of **1** and its tetra-TMSi derivative (**1b**; Figures 2 and 3). The placement of the THF ring moiety was also established by the close examination of fragments ions in the EI-MS of **1** at *m*/*z* 467, 437, 367, and 279, and its tetra-TMSi derivative (**1b**) at *m*/*z* 683, 653, 511, and 279. Thus, the fragmentation pattern observed showed that the non-adjacent bis-THF ring moiety was located from C-11 to C-14 and C-19 to C-22, respectively (Figures 2 and 3). The relative stereochemistry between C-14 and C-15 was indicated as *threo* by comparing the proton signals at H-14 (δ 3.83) and H-15 (δ 3.40) and carbon signals at C-14 (δ 84.0) and C-15 (δ 75.5) with those of known ACGs squamostanin-D [11]. The proton signals at δ 3.31 (H-18), 3.83 (H-19), 3.83 (H-22), and 3.34 (H-23) in the ^{1}H NMR spectrum of **1** suggested the relative stereochemistry of C-18–C-19 and C-22–C-23 was also *threo*. The ^{1}H and ^{13}C NMR spectral data for the methines and methylenes in the rings suggested the relative configurations of the two THF rings were both *trans* [12,13]. Therefore, the relative stereochemistry across the THF ring and the flanking hydroxyls was assigned as *trans/threo/threo/trans/threo*

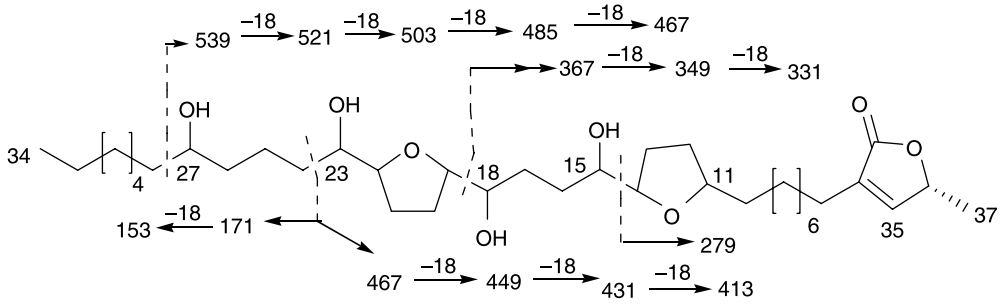


Figure 2. Diagnostic mass fragmentation ions of **1** and **2** (molecular mass = 638 amu) in *m*/*z*, losses of *m*/*z* 18 indicate the loss of H_2O neutrals.

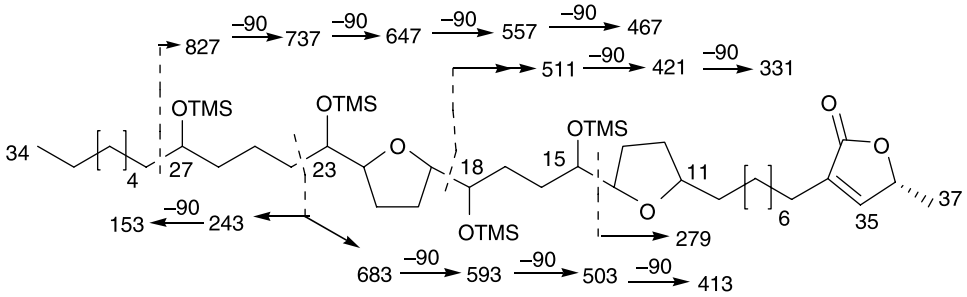


Figure 3. Diagnostic mass fragmentation ions of **1b** and **2b** (molecular mass=926 amu) in *m/z*, losses of *m/z* 90 indicate the loss of TMSiOH neutrals.

which was consistent with that of squamostanin-D. Compound **1** is a structural isomer of squamostanin-D, their difference is the position of the THF ring system, the non-adjacent bis-THF ring of squamostanin-D is located at C-12–C-15 and C-20–C-23, respectively. Thus, the ring system of compound **1** is shifted by one carbon unit toward the γ-lactone ring. To determine the stereochemistry of compound **1**, the (*R*)- and (*S*)-Mosher ester derivatives were prepared, the negative value of δ_H (*S*–*R*) at H-14 (−0.072), H-15 (−0.011), H-17 (−0.017), H-18 (−0.039), H-22 (−0.066), H-23 (−0.054), H-26 (−0.014), and H-27 (−0.044) suggested the absolute stereochemistries were determined as C-15 *R*, C-18 *R*, C-23 *R*, and C-27 *R*, respectively, by using advanced Mosher ester methodology (Table 1). Comparing the NMR spectral data of the MTPA derivatives of **1** with those of model butenolides synthesized by Hoye *et al.* [14], the absolute stereochemistry for C-36 was determined as *S*, which was identical to all the known ACGs. Accordingly, the structure of compound **1** was elucidated as reported in Figure 1.

Compound **2** was obtained as a white wax powder. Its molecular formula was determined to be $C_{37}H_{68}O_8$ by the ion peak at *m/z* 639.4682 $[M + H]^+$ in the HR-EI-MS. The ^1H and ^{13}C NMR spectral data of compound **2** were almost identical to those of compound **1** (Table 3), and the EI-MS of compound **2** was almost superimposable with that of compound **1.** In the ^1H NMR spectrum of **2**, the signals at δ 7.22 (1H, d, H-35), 5.13 (1H, dq, H-36), 2.27 (2H, t, H-3), and 1.37 (3H, d, H-37), together with the carbon signals at δ 172.5 (C-1), 151.8 (C-35), 133.5 (C-2), 79.8 (C-36), 24.3 (C-3), and 19.7 (C-37) also indicated the presence of methyl α,β-unsaturated γ-lactone moiety and the presence of a non-adjacent bis-THF ring system with flanking hydroxyls was also indicated by the ^1H NMR signals at δ 3.80–3.89 (4H, m, H-11, 14, 19, 22), 3.37–3.42 (3H, m, H-15, 18, 27), and the ^{13}C NMR signals at δ 81.3 (C-11), 84.2 (C-14), 84.5 (C-19), 84.0 (C-22), 75.3 (C-15), 74.9 (C-18), and 74.3 (C-27), which showed the two compounds had a similar structure. However, comparing their ^1H and ^{13}C NMR spectra carefully, their main differences were the chemical shifts of H-23 and C-23. In the ^1H NMR spectra of **2** and **2a**, the proton signals at δ 3.82 (1H, m, H-23) and 3.84 (1H, m, H-23), respectively, and the carbon signal at δ 71.3 (C-23), indicated the relative stereochemistry between C-22 and C-23 was *erythro*. So, the relative stereochemistry of THF ring system of compound **2** was *trans/threo/threo/trans/erythro*. The (*R*)- and (*S*)-Mosher ester derivatives of compound **2** were also prepared to determine the absolute configuration and the positive value of δ_H (*S*–*R*) at H-19 (+0.046), H-22 (+0.066), and H-23 (+0.052) suggested an *S*-configuration at C-23 of compound **2**. Thus, the absolute stereochemistries of compound **2** were determined

Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) spectral data for MTPA derivatives of compounds **1** and **2**.

H No.	14	15	16	17	18	19	22	23	24	26	27	28
$\delta 1_S$	3.890	5.006	1.746	1.720	5.108	3.880	3.902	5.300	3.934	1.882	5.120	1.685
$\delta 1_R$	3.962	5.017	1.721	1.737	5.147	3.922	3.968	5.354	3.920	1.896	5.164	1.664
$\delta 1_{S-R}$	−0.072	*R*	+0.025	−0.017	*R*	+0.042	−0.066	*R*	+0.014	−0.014	*R*	+0.021
$\delta 2_S$	3.886	5.106	1.766	1.726	5.168	3.932	3.978	5.300	3.890	1.852	5.070	1.687
$\delta 2_R$	3.925	5.167	1.731	1.737	5.184	3.886	3.912	5.248	3.914	1.879	5.104	1.674
$\delta 2_{S-R}$	−0.039	*R*	+0.035	−0.011	*R*	+0.046	+0.066	*S*	−0.024	−0.027	*R*	+0.013

as C-15 *R*, C-18 *R*, C-23 *S*, and C-27 *R* by using advanced Mosher ester methodology (Table 1). The NMR spectral data of the MTPA derivatives of **2** were compared with those of model butenolides synthesized by Hoye *et al.* [14]. On the basis of the above analysis, the compound **2** is a structural isomer of compound **1** and the structure of compound **2** was elucidated as reported in Figure 1.

The cytotoxicities of compounds **1** and **2** were evaluated against the human tumor cell lines of human colon adenocarcinoma (HCT), human lung carcinoma (A-549), human breast carcinoma (MCF-7), and human prostate adenocarcinoma (PC-3) by the conventional MTT method. The results listed in Table 2 showed that both the compounds exhibited significant selective activities against all four human tumor cell lines in our 2-day MTT tests. As shown in Table 2, compound **2** showed notable selective activity against PC-3 tumor cells with an IC_{50} value 7.33×10^{-4} μg/ml. Furthermore, the result of cytotoxicity bioassays showed compound **2** was generally more bioactive than compound **1**, whose cytotoxicities also exceeded adriamycin. The relative stereochemistry of ACGs may be an important factor for the bioactivity, and the ACGs with *threo/trans/threo/trans/erythro* stereochemical arrangement showed to be gently more active than those with the *threo/trans/threo/trans/threo* stereochemical arrangement. The result of cytotoxicity bioassays also further revealed that the ACGs have a broad range of cytotoxic activity and ACGs have been judged as promising candidates for a future generation of drugs to fight against tumor cells.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco-MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. The UV spectra were taken on a HP 8451A diode

Table 2. Cytotoxicity of **1** and **2** against human tumor cell lines.

	ED_{50} (μg/ml)			
Compound	HCT	A-549	PC-3	MCF-7
1	3.97×10^{-3}	5.67×10^{-3}	5.63×10^{-3}	1.62×10^{-2}
2	3.02×10^{-3}	5.53×10^{-3}	7.33×10^{-4}	1.17×10^{-2}
Adriamycin	4.94×10^{-2}	2.62×10^{-2}	2.87×10^{-1}	1.94×10^{-1}

array spectrophotometer. The IR spectra were recorded on a NEXUS-470 spectrophotometer. The ^1H and ^{13}C NMR spectral data were recorded on Brucker ACF-300P in $CDCl_3$. EI-MS data were obtained on FEOL JMS 300S. HR-EI-MS data were obtained on a PE LC/MS spectrometer. Chromatography was performed on silica gel (200–300 mesh). Optical densities (OD) were read on a BR680 enzyme-labeled detector.

3.2 Plant material

The seeds of *A. squamosa* were collected from Guangdong Province in July 2007 and identified by Prof. Jian-Wei Chen (Nanjing University of Chinese Medicine, Jiangsu, China). The sample was authenticated and is deposited in the Pharmaceutical College of Nanjing University of Chinese Medicine, Jiangsu (No. 083).

3.3 Extraction and isolation

The seeds of *A. squamosa* (14 kg) were pulverized and then extracted three times with 95% EtOH for 2 h at room temperature. The extract (1400 g) was suspended in water and partitioned to provide petroleum ether (180 g), $CHCl_3$ (238 g), and *n*-BuOH (80 g) fractions, respectively. The $CHCl_3$ fraction (208 g) was subjected to Si-gel column chromatography (eluted with $CHCl_3$ and MeOH in increasing polarity) to obtain six fractions (H_1–H_6). The fraction H_2 was further fractioned on Si-gel column chromatography with a step gradient elution of $CHCl_3$–MeOH (100:1, 50:1, 20:1, and 10:1) to afford other 10 fractions (H_7–H_{16}). The fraction H_8 was further subjected to Si-gel column chromatography gradiently eluted with $CHCl_3$–MeOH (50:1, 20:1, 10:1, and 2:1) to afford **1** (52 mg). The H_{10} fraction was also further subjected to Si-gel column chromatography eluted with $CHCl_3$–MeOH (50:1, 20:1, 10:1, and 1:1) to afford **2** (61 mg).

3.3.1 Compound *1*

White amorphous powder; mp 82–84°C; $[\alpha]_D^{25}$ +9.7 ($c = 0.082$, MeOH); UV (MeOH) λ_{max}: 220 nm (log ε 4.27). IR (KBr) ν_{max} (cm^{-1}): 3320, 1720, 1524, and 765. The ^1H and ^{13}C NMR spectral data are reported in Table 3. EI-MS *m/z*: 638, 620, 539, 521, 503, 485, 467, 449, 431, 413, and 279. HR-EI-MS *m/z*: 639.4698 $[M + H]^+$ (calcd for $C_{37}H_{66}O_8$, 639.4758).

3.3.2 Compound *2*

White wax powder; mp 62–63°C; $[\alpha]_D^{25}$ +16.7 ($c = 0.067$, MeOH). UV (MeOH) λ_{max}: 220 nm (log ε 4.20). IR (KBr) ν_{max} (cm^{-1}): 3220, 1715, 1545, and 762. The ^1H and ^{13}C NMR spectral data are reported in Table 3. EI-MS *m/z*: 638, 620, 539, 521, 503, 485, 467, 449, 431, 413, 367, 349, and 279. HR-EI-MS *m/z:* 639.4682 $[M + H]^+$ (calcd for $C_{37}H_{66}O_8$, 639.4758).

3.4 Preparation derivatives

3.4.1 Preparation of tri-TMSi derivatives

Pure compounds **1** and **2** (25 mg) were placed in a 100 ml conical reaction vial, respectively, and dried in a vacuum desiccator over P_2O_5 for 24 h. The sample was treated with 2 μl of pyridine and 20 μl of *N*,*O*-bis(trimethylsilyl)

Table 3. ^{1}H NMR and ^{13}C NMR spectral data of compounds **1** and **2** and their tetra-acetate derivatives **1a** and **2a** (500 MHz, $CDCl_3$, J in Hz and δ in ppm).

H/C No.	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C
	1	**1a**	**1**	**2**	**2a**	**2**
1	–	–	174.5	–	–	172.5
2	–	–	134.5	–	–	133.5
3	2.26 (2H, t, 7.2)	2.22 (2H, t, 7.2)	25.4	2.27 (2H, t, 7.2)	2.24 (2H, t, 7.2)	24.3
4	1.27 (2H, m)	1.17–1.38	27.6	1.23 (2H, m)	1.14–1.33	26.7
5–10	1.26–1.56 (m)	1.22–1.77 (m)	26.3–30.6	1.21–1.55 (m)	1.23–1.76 (m)	25.4–29.7
11	3.93 (1H, m)	4.87 (1H, m)	80.9	3.89 (1H, m)	4.85 (1H, m)	81.3
12	1.96 (2H, m)	2.07 (2H, m)	25.9	1.92 (2H, m)	2.05 (2H, m)	26.1
13	1.97 (2H, m)	2.07 (2H, m)	27.0	1.95 (2H, m)	2.06 (2H, m)	27.7
14	3.83 (1H, m)	3.86 (1H, m)	84.0	3.87 (1H, m)	3.89 (1H, m)	84.2
15	3.40 (1H, m)	4.82 (1H, m)	75.5	3.42 (1H, m)	4.85 (1H, m)	75.3
16	1.74 (2H, m)	1.76 (2H, m)	33.0	1.71 (2H, m)	1.73 (2H, m)	33.2
17	1.72 (2H, m)	1.74 (2H, m)	31.2	1.72 (2H, m)	1.74 (2H, m)	30.8
18	3.31 (1H, m)	4.80 (1H, m)	75.3	3.37 (1H, m)	4.88 (1H, m)	74.9
19	3.83 (1H, m)	3.85 (1H, m)	83.8	3.80 (1H, m)	3.82 (1H, m)	84.5
20	1.92 (2H, m)	1.98 (2H, m)	28.2	1.91 (2H, m)	1.98 (2H, m)	28.7
21	1.95 (2H, m)	1.96 (2H, m)	28.3	1.93 (2H, m)	1.96 (2H, m)	29.1
22	3.83 (1H, m)	3.85 (1H, m)	83.9	3.86 (1H, m)	3.88 (1H, m)	84.0
23	3.34 (1H, m)	4.82 (1H, m)	75.1	3.82 (1H, m)	3.84 (1H, m)	71.5
24	1.74 (2H, m)	1.80 (2H, m)	35.5	1.65 (2H, m)	1.77 (2H, m)	33.6
25	1.77 (2H, m)	1.80 (2H, m)	30.3	1.71 (2H, m)	1.73 (2H, m)	30.1
26	1.75 (2H, m)	1.81 (2H, m)	31.6	1.72 (2H, m)	1.76 (2H, m)	31.5
27	3.40 (1H, m)	4.88 (1H, m)	74.4	3.37 (1H, m)	4.83 (1H, m)	74.3
28	1.73 (2H, m)	1.78 (2H, m)	30.8	1.71 (2H, m)	1.76 (2H, m)	30.6
29	1.43 (2H, m)	1.48 (2H, m)	29.2	1.45 (2H, m)	1.48 (2H, m)	28.7
30	1.42 (2H, m)	1.48 (2H, m)	29.7	1.46 (2H, m)	1.46 (2H, m)	29.6
31	1.41 (2H, m)	1.47 (2H, m)	27.0	1.43 (2H, m)	1.45 (2H, m)	27.3
32	1.42 (2H, m)	1.47 (2H, m)	30.4	1.44 (2H, m)	1.45 (2H, m)	30.6
33	1.36 (2H, m)	1.43 (2H, m)	23.7	1.38 (2H, m)	1.45 (2H, m)	23.2
34	0.90 (3H, t, 7.2)	0.90 (3H, t, 7.2)	14.4	0.93 (3H, t, 7.2)	0.92 (3H, t, 7.2)	14.4
35	7.20 (1H, d, 1.5)	7.24 (1H, d, 1.5)	152.2	7.22 (1H, d, 1.5)	7.25 (1H, d, 1.5)	151.8
36	5.07 (1H, dq, 7.2, 1.5)	5.07 (q, 7.2)	79.5	5.13 (1H, dq, 7.2, 1.5)	5.17 (q, 7.2)	79.8
37	1.38 (3H, d, 7.2)	1.38 (3H, d, 7.2)	19.2	1.37 (3H, d, 7.2)	1.38 (3H, d. 7.2)	19.7
OAC	–	2.06 (6H, S)	–	–	2.06 (6H, S)	–
	–	2.08 (6H, S)	–	–	2.08 (6H, S)	–

acetamide and heated at 70°C for 30 min to yield the tetra-TMSi derivatives (**1b** and **2b**).

*3.4.2 Preparation of per-*S- *and per-*R-*Mosher esters*

Purified compounds **1** and **2** (2.0 mg) were dissolved in 0.5 ml of CH_2Cl_2 and sequentially, 0.2 ml of pyridine, 0.2 mg of 4-(dimethylamino) pyridine, and 25 mg of (*R*)-(−)-α-methoxy-(trifloromethyl) phenylacetyl chloride were added. The mixture was left at room temperature for 4 h and purified over a microcolumn (0.66 cm) of Si-gel eluted with 2 ml of CH_2Cl_2. The eluate was washed with 1% $NaHCO_3$ (5 ml) and H_2O (2 × 5 ml) and dried *in vacuo* to give the *S*-Mosher esters. Use of (*S*)-(+)α-methoxy-α-(trifloromethyl) phenylacetyl chloride gave the *R*-Mosher ester derivatives of compounds **1** and **2**.

3.4.3 Preparation of acetate derivatives

Pure compounds **1** (10 mg) and **2** (12 mg) were dissolved in 0.5–1.0 ml of pyridine, respectively. Anhydrous Ac_2O (1 ml) was added and the mixture was left at room temperature for 4–8 h. The mixture was then partitioned between H_2O and CH_2Cl_2, and the organic layer was concentrated *in vacuo* to afford the pure acetate derivatives (**1a** and **2a**).

3.5 Cytotoxicity assays

Compounds **1** and **2** were assayed for cytotoxicity against HCT, human lung carcinoma (A-549), human breast carcinoma (MCF-7), and human prostate adenocarcinoma (PC-3) with adriamycin as positive standard using MTT method. Freshly trypsinized cell suspensions were seeded in a 96-well microtiter plate at a density of 6000–10,000 cell/ml, and different concentrations of compounds **1** and **2** stocks were added after 24 h seeding.

The microtiter plates were incubated for 48 h in a humidified atmosphere of 5% CO_2 at 37°C and the cellular viability was determined using a standard MTT method, the OD were read on an enzyme-labeled detector (BR680) at a wavelength of 490 nm.

Acknowledgements

The authors acknowledged Prof. Jian-Wei Chen for identifying the seed of *A. squamosa* and Prof. Ming-Yyan Wang for cytotoxicity test guidance.

References

[1] M.C. Zafra-Polo, M.C. González, E. Estornell, S. Sahpaz, and D. Cortes, *Phytochemistry* **42**, 253 (1996).
[2] M.C. Zafra-Polo, B. Figadère, T. Gallardo, J.R. Tormo, and D. Cortes, *Phytochemistry* **48**, 1087 (1998).
[3] L. Zeng, Q. Ye, N.H. Oberlies, G. Shi, Z.M. Gu, K. He, and J.L. McLaughlin, *Nat. Prod. Rep.* **13**, 275 (1996).
[4] F.Q. Alali, X.X. Liu, and J.L. McLaughlin, *J. Nat. Prod.* **62**, 504 (1999).
[5] J.R. Tormo, T. Gallardo, M.C. González, A. Bermejo, N. Cabedo, I. Andreu, and E. Estornell, *Curr. Top. Phytochem.* **2**, 69 (1999).
[6] A. Yazbak, S.C. Sinha, and E. Keinan, *J. Org. Chem.* **63**, 5863 (1998).
[7] F.Q. Alali, L. Rogers, Y. Zhang, J.L. McLaughlin, F.Q. Alali, L. Rogers, and J.L. McLaughlin, *J. Nat. Prod.* **21**, 36 (1999).
[8] M. Londershausen, W. Leicht, F. Lieb, H. Moeschler, and H. Weiss, *Pestic. Sci.* **33**, 427 (1991).
[9] M. Degli Esposti, A. Ghelli, M. Ratta, D. Cortes, and E. Estornell, *J. Biochem.* **161**, 301 (1994).
[10] M.C. Zafra-Polo, M.C. Gonzalez, E. Estornell, S. Sahpaz, and D. Cortes, *Phytochemistry* **42**, 253 (1996).
[11] R.Z. Yanf, X.C. Zheng, S.J. Wu, W.X. Yi, and X.H. Hui, *Act. Botanic. Yunanica* **21**, 517 (1999).
[12] M.H. Woo, K.Y. Cho, Y. Zhang, L. Zeng, Z.M. Gu, and J.L. Mclaughlin, *J. Nutr. Prod.* **58**, 1533 (1998).
[13] G.X. Zhao, Z.M. Gu, L. Zeng, J.F. Chao, J.F. Kozlowski, K.V. Wood, and J.L. Mclaughlin, *Tetrahedron* **51**, 7149 (1995).
[14] T.R. Hoye, P.R. Hanson, L.E. Hasenwinkel, E.A. Ramirez, and Z.P. Zhuang, *Tetrahedron Lett.* **35**, 8529 (1994).