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Cytotoxic Bistetrahydrofuran Annonaceous Acetogenins from the Seeds of *Annona squamosa*

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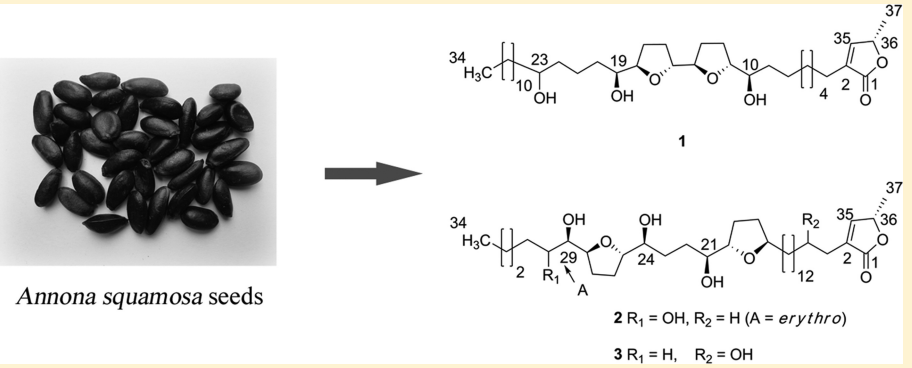
Supporting Information



ABSTRACT: Three new bistetrahydrofuran annonaceous acetogenins (**1**–**3**) were isolated from a 95% EtOH extract of *Annona squamosa* seeds. Four known annonaceous acetogenins, uvarigrandin A (**4**), bullatacin (**5**), squamostatin-A (**6**), and squamostatin-D (**7**), were also isolated. Their structures were elucidated by spectroscopic analyses, and the absolute configurations of the carbinol centers of **1**–**3** were defined by application of the Mosher method. These compounds all exhibited significant cytotoxic activity in vitro against five human tumor cell lines.

Annonaceous acetogenins are reported to exhibit cytotoxic, immunosuppressive, pesticidal, antiparasitic, antimicrobial, and antioxidant activities.[1−5] The potential for acetogenins to inhibit tumor cells that are multiple drug resistant has attracted increasing interest. In the absence of a complete structural model of the inhibitor binding site of complex I, it is difficult to predict the inhibitory potency of new acetogenins.[3] Mechanism of action studies have shown that annonaceous acetogenins inhibit HIF-1 activation by blocking the hypoxic induction of nuclear HIF-1α protein.[6]

As a part of our continued structure−activity investigation of annonaceous acetogenins,[7] three new and four known bistetrahydrofuran acetogenins were isolated from a 95% EtOH extract of *Annona squamosa* Linn. (Annonaceae) seeds. Their structures were elucidated by spectroscopic analyses, and the relative configurations of the tetrahydrofuran rings were established by comparing NMR data with model compounds. Absolute configurations at the carbons bearing OH groups were defined by application of the Mosher method.

Compound **1**, a white powder, gave a molecular formula of $C_{37}H_{66}O_7$ as deduced from HRESIMS (m/z 645.4718 $[M + Na]^+$, calcd 645.4706), indicating five degrees of unsaturation. IR absorptions at 3422 and 1742 cm^{-1}, the UV maximum at 215 nm, and a positive reaction to Kedde's reagent implied the presence of an α,β-unsaturated γ-lactone. Signals in the ^1H NMR spectrum (Table 1) at δ_H 6.97 (H-35), 4.98 (H-36), 1.40 (H-37), and 2.26 (H-3) and in the ^{13}C NMR spectrum (Table 1) at δ_C 173.8 (C-1), 134.4 (C-2), 148.7 (C-35), 77.7 (C-36), and 19.2 (C-37) confirmed the terminal lactone fragment and the absence of an OH group at C-4.[8] The signals at δ_H 3.40 (H-10) and 3.80−3.94 and those at δ_C 83.3 (C-11), 82.8 (C-18), 82.4 (C-14), 82.1 (C-15), 74.1 (C-10), and 71.6 (C-19) in the ^1H and ^{13}C NMR spectra are characteristic of the presence of two adjacent bistetrahydrofuran rings flanked by OH groups.[9] A third OH appeared in the ^1H NMR spectrum at δ_H 3.60 (H-23) and in the ^{13}C NMR at δ_C 71.8 (C-23). This OH group was located at C-23 from observation of the EIMS fragment ions at m/z 155 and 399. The bis-THF unit with flanking OH groups was placed at C-10 to C-19 according to the EIMS fragment ion peaks at m/z 195, 221, 239, 295, and 347 (Figure 1). The relative stereochemistry from C-10 to C-19 of **1** was determined to be *threo/trans/threo/trans/erythro* by careful comparison of ^1H and ^{13}C NMR data with a series of bullatacin-type compounds.[10−14]

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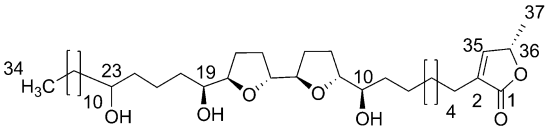
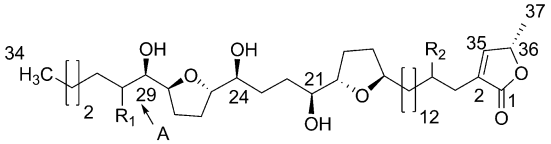
Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−3 Recorded in $CDCl_3$[a]

	1		2		3	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		173.8		173.8		174.7
2		134.4		134.5		131.2
3	2.26, t (7.5)	25.2	2.26, t (7.5)	25.2	2.42, m, 2.53, d (15.0)	33.4
4	1.39−1.43, m	27.4	1.28−1.72, m	27.5	3.78−3.88, m	70.0
5	1.26, s	22.0−29.7	1.28−1.72, m	22.0−32.4	1.26−1.73, m	37.4
6−8	1.26, s	22.0−29.7	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
9	1.39−1.43, m	33.4	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
10	3.40, m	74.1	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
11	3.80−3.94, m	83.3	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
12	1.64, m, 1.93, m	28.4	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
13	1.64, m, 1.93, m	28.9	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
14	3.80−3.94, m	82.4	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
15	3.80−3.94, m	82.1	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
16	1.64, m, 1.93, m	28.9	1.28−1.72, m	35.7	1.26−1.73, m	35.6
17	1.64 m, 1.93, m	25.6	3.76−3.91, m	79.4	3.78−3.88, m	79.3
18	3.80−3.94, m	82.8	1.88, m, 2.00, m	32.7	1.86−2.00, m	32.5
19	3.80−3.94, m	71.6	1.88, m, 2.00, m	28.5	1.86−2.00, m	28.4
20	1.39−1.43, m	31.8	3.76−3.91, m	82.0	3.78−3.88, m	82.0
21	1.39−1.43, m	32.6	3.41, m	74.5	3.41, m	74.5
22	1.39−1.43, m	37.5	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
23	3.60, m	71.8	1.28−1.72, m	22.0−32.4	1.26−1.73, m	25.2−29.7
24	1.39−1.43, m	37.3	3.41, m	74.6	3.41, m	74.6
25	1.26, s	22.0−29.7	3.76−3.91, m	83.4	3.78−3.88, m	83.3
26	1.26, s	22.0−29.7	1.88, m, 2.00, m	28.6	1.86−2.00, m	28.6
27	1.26, s	22.0−29.7	1.88, m, 2.00, m	25.6	1.86−2.00, m	25.5
28	1.26, s	22.0−29.7	3.76−3.91,m	82.3	3.78−3.88, m	82.2
29	1.26, s	22.0−29.7	3.76−3.91,m	71.8	3.78−3.88, m	71.5
30	1.26, s	22.0−29.7	3.59, m	71.9	3.78−3.88, m	32.4
31	1.26, s	22.0−29.7	1.28−1.72, m	37.4	1.26−1.73, m	26.0
32	1.26, s	31.8	1.28−1.72, m	22.0−32.4	1.26−1.73, m	31.9
33	1.39−1.43, m	22.6	1.28−1.72, m	22.0−32.4	1.26−1.73, m	22.7
34	0.88, t (7.0)	14.0	0.88, t (7.0)	14.1	0.88, t (6.8)	14.1
35	6.97, td (1.5, 1.5, 1.5)	148.7	6.96, td (1.5, 1.5, 1.5)	148.8	7.12, s	151.9
36	4.98, qdd (6.8, 1.5, 1.5)	77.7	4.98, qdd (6.5, 1.5, 1.5)	77.0	5.06, q (6.5)	78.0
37	1.40, d (6.8)	19.2	1.40, d (6.5)	19.3	1.43, d (6.5)	19.1

[a] δ from TMS (ppm). Assignments confirmed by HSQC experiments. ^{1}H NMR [500 MHz, $CDCl_3$, J (Hz)] and ^{13}C NMR (500 MHz) spectroscopic data for compounds **1** and **2**. ^{1}H NMR [300 MHz, $CDCl_3$, J (Hz)] and ^{13}C NMR (300 MHz) spectroscopic data for compound **3**.



1



2 R_1 = OH, R_2 = H (A = *erythro*)

3 R_1 = H, R_2 = OH

Compound **2** had the molecular formula $C_{37}H_{66}O_8$ as determined by HRESIMS at m/z 661.4664 $[M + Na]^+$, calcd 661.4655. IR absorption bands at 3424 and 1740 cm^{-1} indicated the presence of OH and carbonyl groups. The presence of two nonadjacent bistetrahydrofuran rings with three flanking OH groups was indicated by ^{1}H NMR signals at δ_H 3.41 (H-21, 24) and 3.76−3.91 and by ^{13}C NMR signals at δ_C 83.4 (C-25), 82.3 (C-28), 82.0 (C-20), and 79.4 (C-17) (Table 1). The OH groups in **2** were placed at C-21, C-24, and C-29 on the basis of the fragment ions in the EIMS at m/z 81, 161, 275, 293, 327, 345, 363, 415, and 433 (Figure 1). The fourth OH group appeared in the ^{1}H NMR spectrum at δ_H 3.59 (H-30) and ^{13}C NMR at δ_C 71.9 (C-30). This OH group was located at C-30 on the basis of the EIMS fragment ions at m/z 55 and 67 (Figure 1).

Compound **3** was determined to have the molecular formula $C_{37}H_{66}O_8$ from the ion peak (m/z 661.4664 $[M + Na]^+$, calcd 661.4655) in the HRESIMS. The UV and IR data indicated the presence of OH groups, a carbonyl, and a double bond. The NMR data (Table 1) was consistent with an α,β-unsaturated γ-lactone and nonadjacent bistetrahydrofuran rings with three flanking hydroxyls. The EIMS fragment at m/z 141 (Figure 1) was characteristic of a terminal methylated α,β-unsaturated γ-lactone with a 4-OH group. The other OH groups in **3** were assigned to C-21, C-24, and C-29 on the basis of the fragment

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ions in the EIMS at *m/z* 81, 161, 309, 361, 379, 413, 431, 449, and 491 (Figure 1). The configuration at C-29/C-30 in **2** was concluded to be *erythro*, and the relative configuration across the THF ring and the flanking hydroxyls of **2** and **3** was assigned as *trans/threo–threo/trans/erythro* by comparison of ^{1}H and ^{13}C NMR data with a series of bullatalicin-type compounds.[15–17]

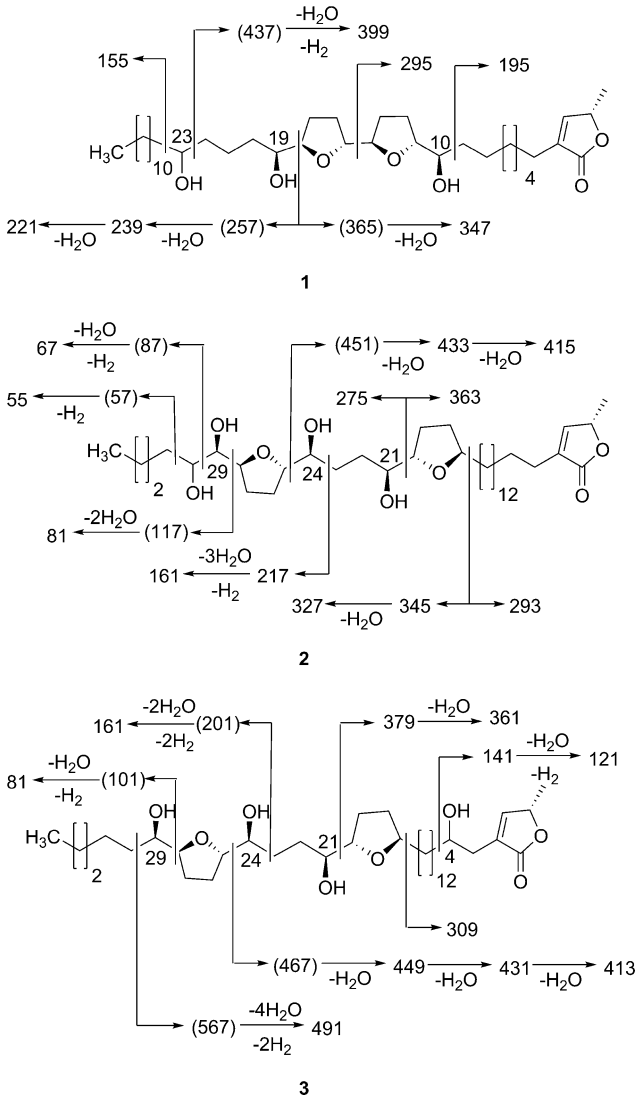

Figure 1. Diagnostic EIMS fragment ions (*m/z*) of **1–3**. Peaks in parentheses were not observed.

The absolute configurations of the carbinol centers of compounds **1–3** were determined by analysis of the ^{1}H NMR data of the *S*-Mosher (**1r**, **2r**, and **3r**) and *R*-Mosher (**1s**, **2s**, and **3s**) ester derivatives (Table 1S, Supporting Information). The absolute configurations at C-10 and C-19 in **1** were determined to be *R* and *S*. The *S,S*-1,2 diols of **2** at C-29,30 were determined from the chemical shifts that appeared at δ_H 5.00 and 5.08 in the *S*-Mosher ester and at δ_H 5.01 and 5.19 in the *R*-Mosher ester.[18] The absolute configurations of C-21, C-24, C-29, and C-30 in **2** were determined to be *S*, *R*, *S*, and *S*. The $\Delta\delta_H$ values for H-35 and H-36 in **3r** and **3s** at 0.25 and 0.03 suggested that **3** had a 4*R* configuration.[19,20] Therefore, the absolute configurations at C-4, C-21, C-24, and C-29 in **3** were determined to be *R*, *S*, *R*, and *S*, respectively. The absolute configuration at C-36 of **1–3** was assumed to be *S* since this chiral center has been determined to be *S* in most of the acetogenins that have been reported.[21–24] We named compound **1** annosquacin-I, **2** annosquatin-I, and **3** annosquatin-II.

Known compounds were characterized as uvarigrandin A (**4**),[13,25] bullatacin (**5**),[26] squamostatin-A (**6**),[15] and squamostatin-D (**7**)[27] by comparison of their spectroscopic data with published values. The structures of compounds **4–7** are included in the Supporting Information.

Bioactivity data obtained with compounds **4–7** are summarized in Table 2. They all showed cytotoxicity comparable to fluorouracil for the human lung carcinoma (A-549), cervix carcinoma (HeLa), breast carcinoma (MCF-7), hepatoma carcinoma (HepG2 and SMMC-7721), and gastric adenocarcinoma (MKN-45) cell lines. Bioactivity data showed that adjacent bistetrahydrofuran annonaceous acetogenins annosquacin-I, uvarigrandin A, and bullatacin were more cytotoxic than nonadjacent bistetrahydrofuran annonaceous acetogenins squamostatin-A, squamostatin-D, annosquatin-I, and annosquatin-II. A free OH at C-4 seems to increase cytotoxicity of acetogenins. As examples, bullatacin and annosquatin-II were more active than uvarigrandin A and annosquatin-I. The tetrahydroxylated nonadjacent bistetrahydrofuran annonaceous acetogenin squamostatin-A was less cytotoxic than trihydroxylated squamostatin-D. This is in accord with earlier observations.[28–30]

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a YanacoMP-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 array spectrophotometer. IR spectra were recorded on a

Table 2. Cytotoxic (IC_{50}) Values of Compounds 1–7 against Human Tumor Cell lines

	IC_{50} (μg/mL)					
compound	A-549[a]	HeLa[b]	MCF-7[c]	HepG2[d]	SMMC-7721[d]	MKN-45[e]
1, annosquacin-I	1.2×10^{-2}	2.5×10^{-2}	5.2×10^{-2}	2.2×10^{-2}	5.7×10^{-2}	4.2×10^{-2}
2, annosquatin-I	3.1×10^{-1}	5.7×10^{-1}	5.0×10^{-1}	1.7×10^{-1}	1.1×10^{-1}	1.4×10^{-1}
3, annosquatin-II	6.8×10^{-1}	1.5	7.5×10^{-1}	8.0×10^{-1}	3.9×10^{-1}	5.9×10^{-1}
4, uvarigrandin A	8.0×10^{-2}	7.0×10^{-2}	9.6×10^{-2}	7.8×10^{-1}	2.9×10^{-3}	6.9×10^{-2}
5, bullatacin	3.3×10^{-2}	3.4×10^{-2}	4.3×10^{-2}	3.2×10^{-2}	4.8×10^{-3}	5.3×10^{-2}
6, squamostatin-A	2.1×10^{-1}	4.5×10^{-1}	1.5×10^{-1}	8.3×10^{-1}	4.9×10^{-1}	9.3×10^{-1}
7, squamostatin-D	4.6×10^{-2}	1.0×10^{-1}	1.1×10^{-1}	5.6×10^{-1}	2.5×10^{-1}	8.7×10^{-1}
fluorouracil[f]	3.2×10^{-1}	1.4×10^{-1}	2.2×10^{-1}	8.2×10^{-3}	2.3×10^{-2}	3.6×10^{-1}

[a]Human lung carcinoma. [b]Human cervix carcinoma. [c]Human breast carcinoma. [d]Human hepatoma carcinoma. [e]Human gastric adenocarcinoma. [f]Positive control standard.

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NEXUS-470 spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker ACF-300P or Bruker ACF-500P spectrometers in $CDCl_3$. Exact masses (HRESIMS) were measured using a Waters Synapt HDMS system. Chromatography was performed on silica gel (200−300 mesh) (Qingdao Haiyang Chemical Co., Ltd., People's Republic of China). Samples were purified on an Agilent 1200 HPLC system with a G1315D DAD detector equipped with a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm).

Plant Material. The seeds of *A. squamosa* were collected from Guangdong Province in July 2007 and identified by one of the authors (J.-W.C.). The sample was authenticated and was deposited in the Pharmaceutical College of Nanjing University of Chinese Medicine, Jiangsu (No. 083).

Bioassays. Five-day in vitro MTT cytotoxicity tests[31,32] against human tumor cell lines were carried out at the Jiangsu Key Laboratory for Pharmacology and Safety Evaluation of Chinese Materia Medica, using A-549 (human lung carcinoma), HeLa (human cervix carcinoma), MCF-7 (human breast carcinoma), HepG2 and SMMC-7721 (human hepatoma carcinoma), and MKN-45 (human gastric adenocarcinoma) cell lines, with fluorouracil as a positive control.

Extraction and Isolation. The seeds of *A. squamosa* (10.0 kg) were extracted and partitioned, as previously described,[33] to obtain a $CHCl_3$-soluble fraction. The $CHCl_3$ fraction (408 g) was subjected to open Si gel column chromatography (200−300 mesh, 4.0 kg) and eluted with a gradient of increasing polarity [petroleum ether−EtOAc (100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 2:1, 1:1 to pure EtOAc), then MeOH]. The 400 fractions collected were then combined according to their TLC patterns to obtain 10 fractions (F1−F10). The solids obtained from fractions F7 and F8 were washed with petroleum ether to yield squamostatin-D (**7**, 37 mg) and squamostatin-A (**6**, 121 mg), repectively. Fraction F9 (52 g) was further separated with a step gradient elution of petroleum ether−EtOAc (5:1, 2:1, 1:1 to pure EtOAc) and EtOAc−MeOH (20:1, 10:1, 5:1, 2:1, 1:1 to pure MeOH) to afford fractions F11−F20. The residue from F14 (eluted with pure EtOAc, 350 mg) was further separated using HPLC on a YMC-Pack ODS-A column (MeOH−H_2O, 95:5; 1.5 mL/min and 220 nm detection) to yield **1** (22 mg),. The materials from fractions F15 (eluted with EtOAc−MeOH, 20:1) and F16 (eluted with EtOAc−MeOH, 10:1) were washed with petroleum ether to yield **2** (74 mg) and **3** (15 mg), respectively. The materials contained in F17−20 (eluted with EtOAc−MeOH, 5:1, to pure MeOH, 420 mg) were further purified by HPLC (YMC-Pack ODS-A column; MeOH−H_2O, 95:5, 1.5 mL/min and 220 nm detection) to yield uvarigrandin A (**4**, 22 mg) and bullatacin (**5**, 18 mg).

*Annosquacin-I (**1**):* white powder (22 mg); mp 56−57 °C; $[\alpha]^{25}_D$ + 3.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.75) nm; IR (KBr) ν_{max} 3422, 2920, 2851, 1742, and 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS (positive-ion mode) *m*/*z* 645.4718 $[M + Na]^+$ (calcd for $C_{37}H_{66}O_7$, 645.4706).

*Annosquatin-I (**2**):* white powder (75 mg); mp 63−64 °C; $[\alpha]^{25}_D$ + 15.7 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.24) nm; IR (KBr) ν_{max} 3425, 2923, 2853, 1740, and 1063 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z* 661.4665 $[M + Na]^+$ (calcd for $C_{37}H_{66}O_8$, 661.4655).

*Annosquatin-II (**3**):* white powder (15 mg); mp 60−61 °C; $[\alpha]^{25}_D$ + 7.7 (*c* 0.67, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.82) nm; IR (KBr) ν_{max} 3427, 2920, 2850, 1747, and 1065 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z* 661.4664 $[M + Na]^+$ (calcd for $C_{37}H_{66}O_8$, 661.4655).

Preparation of Mosher Esters. To a solution of an acetogenin (1.0 mg in 0.5 mL of CH_2Cl_2) were sequentially added pyridine (0.4 mL), 4-(dimethylamino)pyridine (0.5 mg), and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (5 μL). The mixture was stirred at room temperature for 3 h and filtered through a disposable pipet (0.6 × 6 cm) containing silica gel (200 mesh) eluted with 5 mL of CH_2Cl_2. The CH_2Cl_2 residue was dried in vacuo to give the *S*-Mosher esters. Using (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride gave the *R*-Mosher ester.[12,34]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The structures of known compounds **4**−**7** and spectroscopic data for compounds **1**−**3**, **1r**−**3r**, and **1s**−**3s** (Table 1S) are available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Yazbak, A.; Sinha, S. C.; Keinan, E. *J. Org. Chem.* **1998**, *63*, 5863−5868.
(2) Liaw, C. C.; Wu, T. Y.; Chang, F. R.; Wu, Y. C. *Planta Med.* **2010**, *76*, 1390−1404.
(3) Bermejo, A.; Figadere, B.; Zafra-Polo, M. C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269−303.
(4) Lima, L. A. R.; Pimenta, L. P. S.; Boaventura, M. A. D. *Food Chem.* **2010**, *122*, 1129−1138.
(5) McLaughlin, J. L. *J. Nat. Prod.* **2008**, *71*, 1311−1321.
(6) Coothankandaswamy, V.; Liu, Y.; Mao, S. C.; Morgan, J. B.; Mahdi, F.; Jekabsons, M. B.; Nagle, D. G.; Zhou, Y. D. *J. Nat. Prod.* **2010**, *73*, 956−961.
(7) Yang, H. J.; Zhang, N.; Li, X.; Chen, J. W.; Cai, B. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2199−2202.
(8) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z. M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306.
(9) Chang, F. R.; Wu, Y. C. *J. Nat. Prod.* **2001**, *64*, 925−931.
(10) Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163−1174.
(11) Etcheverry, S.; Sahpaz, S.; Fall, D.; Laurens, A.; Cavé, A. *Phytochemistry* **1995**, *38*, 1423−1426.
(12) Chávez, D.; Mata, R. *Phytochemistry* **1999**, *50*, 823−828.
(13) Hisham, A; Pieters, L. A. C.; Claeys, M.; Van den Heuvel, H.; Esmans, E.; Dommisse, R.; Vlietinck, A. J. *Phytochemistry* **1991**, *30*, 2373−2377.
(14) Araya, H.; Sahai, M.; Singh, S.; Singh, A. K.; Yoshida, M.; Hara, N.; Fujimoto, Y. *Phytochemistry* **2002**, *61*, 999−1004.
(15) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175−1184.
(16) Liu, X. X.; Alali, F. Q.; Hopp, D. C.; Rogers, L. L.; Pilarinou, E.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1998**, *6*, 959−965.
(17) Nonfon, M.; Lieb, F.; Moeschler, H.; Wendisch, D. *Phytochemistry* **1990**, *29*, 1951−1954.
(18) Zeng, L.; Wu, F. E.; Gu, Z. M.; McLaughlin, J. L. *Tetrahedron Lett.* **1995**, *36*, 5291−5294.
(19) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8525−8528.
(20) Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Raminez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8529−8532.

(21) Gu, Z. M.; Zhao, G. X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; pp 249−310.
(22) Liaw, C. C.; Yang, Y. L.; Chen, M.; Chang, F. R.; Chen, S. L.; Wu, S. H.; Wu, Y. C. *J. Nat. Prod.* **2008**, *71*, 764−771.
(23) Zhou, G. X.; Chen, R. Y.; Zhang, Y. J.; Yu, D. Q. *J. Nat. Prod.* **2000**, *63*, 1201−1204.
(24) Kim, D. H.; Ma, E. S.; Suk, K. D.; Son, J. K.; Lee, J. S.; Woo, M. H. *J. Nat. Prod.* **2001**, *64*, 502−506.
(25) Pan, X. P.; Yu, D. Q. *Acta. Pharm. Sin.* **1997**, *32*, 286−293.
(26) Hui, Y. H.; Rupprecht, J. K.; Liu, Y. M.; Anderson, J. E.; Smith, D. L.; Chang, C. J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463−477.
(27) Hirayama, K.; Akashi, S.; Yuji, R.; Niitsu, U.; Fujimoto, Y. *Org. Mass Spectrom.* **1993**, *28*, 1516−1524.
(28) Chavez, D.; Mata, R. *J. Nat. Prod.* **1998**, *61*, 580−584.
(29) Chang, F. R.; Chen, J. L.; Lin, C. Y.; Chiu, H. F.; Wu, M. J.; Wu, Y. C. *Phytochemistry* **1999**, *51*, 883−889.
(30) Chang, F. R.; Liaw, C. C.; Lin, C. Y.; Chou, C. J.; Chiu, H. F.; Wu, Y. C. *Planta. Med.* **2003**, *69*, 241−246.
(31) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(32) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Currents, M. R.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827−4833.
(33) Yang, H. J.; Li, X.; Zhang, N.; Chen, J. W.; Wang, M. Y. *J. Asian Nat. Prod. Res.* **2009**, *11*, 250−256.
(34) Gu, Z. M.; Zeng, L.; Schwedler, J. T.; Wood, K. V.; Mclaughlin, J. L. *Phytochemistry* **1995**, *40*, 467−477.