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Oleanane-Type Triterpenoids from the Leaves and Twigs of *Fatsia polycarpa*

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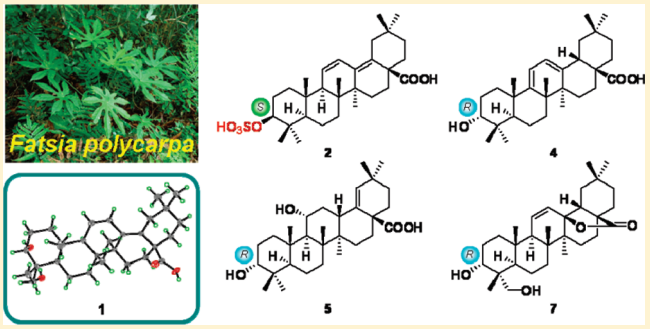
Supporting Information

ABSTRACT: Seven new oleanane-type triterpenoids (**1**−**7**), named fatsicarpains A−G, and the known compounds 3α-hydroxyolean-11,13(18)-dien-28-oic acid (**8**) and 3α-hydroxyolean-11-en-28,13β-olide (**9**) were isolated from the leaves and twigs of *Fatsia polycarpa* on the basis of bioassay-guided fractionation. The structures of compounds **1**−**7** were elucidated through spectroscopic analyses and single-crystal X-ray crystallography of **1**, **8**, and **9**. Cytotoxicity against HepG2 2.2.15 and AGS cells and antihepatitis B virus (HBV) and antibacterial activities of **1**−**9** were also evaluated in vitro.



Plants of the genus *Fatsia* (Araliaceae) are endemic to the Archipelago of the western Pacific rim and Bonin Islands.[1] There are three known *Fatsia* species: *F. japonica*, *F. oligocarpella*, and *F. polycarpa*.[1] Flowers, mature fruits, and leaves of *F. japonica* are rich in triterpeniod saponins.[2−11] Saponins isolated from *F. japonica* have been recommended for treatment of ankylosing spondyloarthritis, osteoarthritis, rheumatism, rheumatoid arthritis with accompanying reactive gout, osteochondrosis, synovitis, and tendinitis.[10] They may also be helpful as a cofactor in antiaging cosmetics and in protein therapy for disorders related to the antioxidant enzymes Cu, Zn-SOD or reactive oxygen species.[11] *Fatsia polycarpa* Hayata (Araliaceae) is an evergreen shrub found in moist shady forests at middle elevations (2000−2800 m) in Taiwan.[12] Our search for bioactive constituents prompted us to investigate secondary metabolites of this plant that not been reported previously.

Dichloromethane, EtOAc, and *n*-BuOH extracts of *F. polycarpa* were assayed for cytotoxicity against HepG2 2.2.15 (human hepatocellular carcinoma) cells. The active compounds isolated from the CH_2Cl_2 extract were purified and structurally identified. Seven new oleanane-type triterpenoids (**1**−**7**) and two known analogues, 3α-hydroxyolean-11,13(18)-dien-28-oic acid (**8**)[13] and 3α-hydroxyolean-11-en-28,13β-olide (**9**),[13] were obtained. The structures of **1**−**7** were elucidated using extensive spectroscopic analyses and by comparison with data reported in the literature. The structures of **1**, **8**, and **9** were confirmed by X-ray diffraction analysis.[14]

Some oleanane-type compounds are of interest due to their antitumor, anti-HIV, anti-inflammation, antioxidation, antihyperglycemia, and cardiovascular properties.[15] Of four million patients worldwide infected with hepatitis B virus (HBV), about 20% are expected to develop chronic hepatitis, liver cirrhosis, or hepatocarcinoma.[16] *Helicobacter pylori* infection is associated with an increased risk for development of duodenal ulcers, gastric ulcers, gastric adenocarcinomas, and gastric lymphomas. However, antibiotic resistance to *H. pylori* and other bacterial pathogens is an increasing problem for eradicating infection.[17] Therefore, safe and efficient treatments to decrease the need for or to replace antibiotics for eradicating *H. pylori* and other infections are needed. Compounds **1**−**9** were evaluated in vitro for cytotoxicity against cancer cell lines HepG2 2.2.15 and AGS (human gastric cancer epithelial cells). Bioassays were also conducted for antihepatitis B virus and for antibacterial activity against *H. pylori*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

RESULTS AND DISCUSSION

Conventional extraction procedures were used, and a MeOH extract of the leaves and twigs of *F. polycarpa* was suspended in H_2O and then partitioned successively with CH_2Cl_2, EtOAc, and *n*-BuOH. Chromatographic separation of the CH_2Cl_2-soluble fraction using normal-phase Si-60, reversed-phase C_{18} gel, and Sephadex LH-20 columns in combination with semipreparative reversed-phase C_{18} HPLC led to the purification of oleanane-type triterpenoids **1**−**9**.

Compound **8** has been previously isolated from the aerial parts of the Argentinean shrub *Junellia tridens*.[13] Definitive support for

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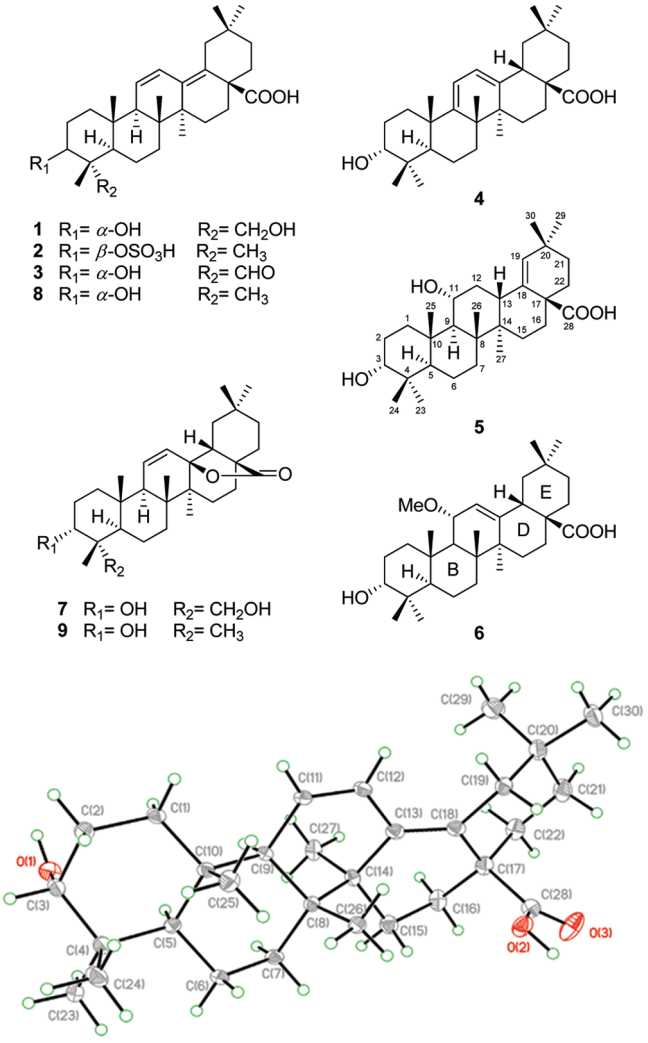

Figure 1. X-ray ORTEP diagram of compound **8**.

the proposed structure of **8** was provided by X-ray crystallographic analysis (Figure 1).[14] The HRESIMS (*m*/*z* 469.3324 $[M - H]^-$) of **1** established a molecular formula of $C_{30}H_{46}O_4$, requiring eight degrees of unsaturation. The analysis of COSY, HMBC, and HSQC spectra permitted us to assign all the spectroscopic signals and to propose the structure for **1**. It possessed an oleanane-type triterpene skeleton similar to that of **8**. The major differences were the chemical shifts of a hydroxymethyl moiety [δ_H 3.55 (1H, d, J = 11.4 Hz) and 3.43 (1H, d, J = 11.4 Hz); δ_C 71.3 (CH_2)] in **1** instead of those of the methyl moiety in **8**. The HMBC spectrum showed correlations from H_2-23 to C-3, C-4, and C-5, supporting the presence of a hydroxy group at C-23. The relative configuration of **1**, assigned by the NOESY spectrum, was compatible with that revealed by computer modeling, in which the close contacts of atoms calculated in space were consistent with the NOESY correlations. It was further identical to the X-ray diffraction analysis of **1** (Figure 2).[14] Accordingly, the structure of 3α,23-dihydroxyolean-11,13(18)-dien-28-oic acid was characterized as fatsicarpain A.

The HRESIMS (*m*/*z* 533.2945 $[M - H]^-$) of **2** indicated its molecular formula to be $C_{30}H_{46}O_6S$. Other prominent ions at *m*/*z* 488 $[M - COOH - H]^-$ and 437 $[M - OSO_3H - H]^-$ in the LRESIMS supported the molecular formula. The NMR data

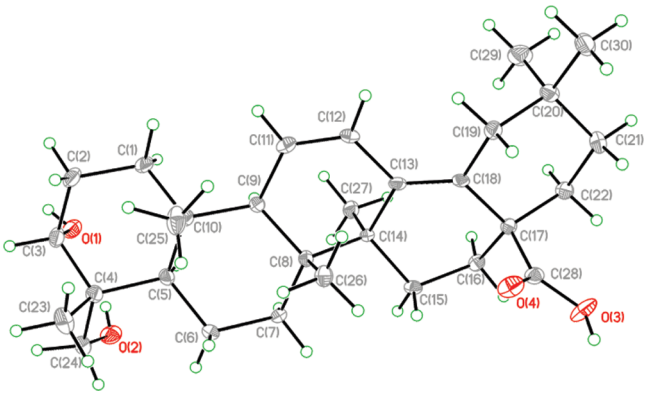

Figure 2. X-ray ORTEP diagram of compound **1**.

(Tables 2 and 3) of **2** were analogous to those of **8**, except for NMR signals due to a sulfate group in **2** and the corresponding downfield shift of the C-3 signals [δ_H 3.96 (1H, dd, J = 12.0, 4.8 Hz) and δ_C 87.5 (CH)] in **2**.[18,19] Furthermore, a strong IR absorption at 1216 cm^{-1} also suggested that **2** possessed a sulfate group at C-3,[18,19] which was identified by the COSY correlations between H_2-1/H_2-2 and H_2-2/H-3 and the HMBC cross-peaks from H_3-23/H_3-24 to C-3 and from H-3 to C-1. Acid hydrolysis of **2**, followed by treatment with $BaCl_2$, gave a white precipitate, further confirming the presence of a sulfate residue.[19] The relative configurations of **2** were demonstrated by the NOESY spectrum with the aid of proton−proton coupling constant analysis. H-3 showed NOESY correlations with H-1α, H-2α, and H-5, indicating the *S**-configuration at C-3. This was supported by the large coupling constant ($^3J_{2\beta,3}$ = 12.0 Hz). In addition, the small coupling constant ($^3J_{2\alpha,3}$ = 4.8 Hz) indicated that they should be axial and equatorial oriented, respectively. Hence, fatsicarpain B (**2**) was elucidated as 3β-sulfooxyolean-11,13(18)-dien-28-oic acid.

The HRESIMS (*m*/*z* 467.3156 $[M - H]^-$) of **3** established a molecular formula of $C_{30}H_{44}O_4$, implying nine degrees of unsaturation. An oxygenated methine [δ_H 3.82 (1H, dd, J = 3.0, 2.4 Hz) and δ_C 73.0 (C-3)] implied that an OH was present at C-3, which was supported by HMBC correlations from H-3 to C-1 and C-5. The NMR data (Tables 1 and 3) of **3** showed distinct similarities to **1**, with major differences at C-23. The C-23 hydroxymethyl protons resonated as a doublet at δ_H 3.55 and a doublet at δ_H 3.43 with a carbon shift value of δ_C 71.3 in **1**, whereas the formyl proton of C-23 appeared as a singlet at δ_H 9.62 with a carbon shift of δ_C 209.7 in **3**. Therefore, fatsicarpain C (**3**) was concluded to be 23-formyl-3α-hydroxyolean-11,13(18)-dien-28-oic acid.

The HRESIMS of **4** showed a $[M + Na]^+$ peak at *m*/*z* 477.3313, which analyzed for molecular formula $C_{30}H_{46}O_3$. Compound **4**, with a conjugated diene in the C ring, differed from **8**, possessing a conjugated diene in the C/D ring. NMR data of **4** (Tables 1 and 3) showed the presence of two vinyl proton signals as an AB pair of doublets at δ_H 5.58 and 5.62 (J = 5.4 Hz) coupled with the carbon signals at δ_C 120.7 and 115.2 in the HSQC spectrum, while the two double bonds at C-9/C-11 and C-12/C-13 were confirmed by $^1H-^1H$ COSY and HMBC correlations. The NOESY correlations between H-18 and H-19β implied a *cis* configuration for the D/E ring junction. Consequently, fatsicarpain D (**4**) was assigned as 3α-hydroxyolean-9,12-dien-28-oic acid.

The HRESIMS (*m*/*z* 495.3437 $[M + Na]^+$) of **5** established a molecular formula of $C_{30}H_{48}O_4$, corresponding to seven degrees of unsaturation. Five different structural units were assembled

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Table 1. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds 1, 3, 4, 6, and 7

	1	3	4	6	7
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	α: 1.39, m	α: 1.49, m	α: 1.66, m	α: 1.52, m	α: 1.35, m
	β: 1.65, m	β: 1.69, m	β: 1.71, m	β: 1.58, m	β: 1.59, m
2	α: 1.53, m	α: 1.61, dq (15.0, 3.0)	α: 1.65, m	α: 1.51, m	α: 1.53, m
	β: 2.01, m	β: 2.00, m	β: 2.00, m	β: 1.96, m	β: 2.00, m
3	3.72, dd (3.0, 2.4)	3.82, dd (3.0, 2.4)	3.42, br s	3.39, br s	3.72, br s
5	1.76, br d (10.8)	2.10, dd (12.6, 2.4)	1.29, br d (10.8)	1.24, m	1.69, m
6	α: 1.46, m	α: 1.53, m	α: 1.47, m	α: 1.44, m	α: 1.47, m
	β: 1.00, m	β: 1.16, m	β: 0.92, m	β: 0.91, m	β: 1.52, m
7	α: 1.43, m	α: 1.49, m	α: 1.64, m	α: 1.45, m	α: 1.51, m
	β: 1.34, m	β: 1.32, m	β: 1.32, m	β: 1.20, m	β: 1.23, m
9	2.13, br s	2.22, br s		1.80, d (8.4)	2.08, br s
11	5.67, dd (10.8, 1.8)	5.66, dd (10.2, 1.8)	5.62, d (5.4)	3.80, dd (8.4, 3.0)	6.07, br d (10.2)
12	6.44, dd (10.8, 3.0)	6.45, dd (10.2, 3.0)	5.58, d (5.4)	5.46, d (3.0)	5.42, dd (10.2, 3.0)
15	α: 1.07, m	α: 1.09, m	α: 1.16, m	α: 1.06, m	α: 1.21, m
	β: 1.69, m	β: 1.71, m	β: 1.99, m	β: 1.99, m	β: 1.71, m
16	α: 1.99, m	α: 1.98, m	α: 2.01, m	α: 2.01, m	α: 2.13, m
	β: 1.70, m	β: 1.70, m	β: 1.67, m	β: 1.62, m	β: 1.32, m
18			2.84, dd (14.4, 5.4)	2.84, br d (10.8)	2.07 br d (13.2)
19	α: 1.69, d (13.8)	α: 1.68, d (13.8)	α: 1.58, m	α: 1.61, m	α: 1.81, t (13.2)
	β: 2.53, dd (13.8, 2.4)	β: 2.53, dd (13.8, 2.4)	β: 1.21, m	β: 1.19, m	β: 1.35, m
21	α: 1.39, d (9.6)	α: 1.39, d (9.6)	α: 1.23, m	α: 1.22, m	α: 1.30, m
	β: 1.29, m	β: 1.29, m	β: 1.36, m	β: 1.34, m	β: 1.36, m
22	α: 1.39, d (9.6)	α: 1.39, d (9.6)	α: 1.80, m	α: 1.59, m	α: 1.64, m
	β: 2.27, dd (9.6, 3.0)	β: 2.26, dd (9.6, 3.0)	β: 1.66, m	β: 1.76, m	β: 1.68, m
23	a: 3.55, d (11.4)	9.62, s	0.99, s	0.94, s	a: 3.54, d (11.4)
	b: 3.43, d (11.4)				b: 3.43, d (11.4)
24	0.70, s	1.04, s	0.86, s	0.83, s	0.70, s
25	0.95, s	0.95, s	1.18, s	1.01, s	0.94, s
26	0.80, s	0.80, s	0.98, s	0.75, s	1.07, s
27	1.00, s	1.02, s	1.04, s	1.22, s	1.09, s
29	0.94, s	0.95, s	0.91, s	0.91, s	0.88, s
30	0.80, s	0.80, s	0.95, s	0.94, s	0.97, s

using the ^{1}H−^{1}H COSY spectrum with the assistance of an HMBC experiment (S15, Supporting Information). The HMBC cross-peaks from H_3-23/H_2-24 to C-3, C-4, and C-5, from H_3-25 to C-1, C-5, C-9, and C-10, from H_3-26 to C-7, C-8, C-9, and C-14, from H_3-27 to C-8, C-13, C-14, and C-15, from H_2-16 to C-28, from H-19 to C-13, C-17, C-20, and C-21, and from H_3-29/H_3-30 to C-19, C-20, and C-21 permitted the establishment of the carbon skeleton. The ^{1}H NMR spectrum of **5** (Table 2) showed a signal at δ_H 3.93 (1H, td, J = 10.8, 4.2 Hz) that suggested the presence of an OH at C-11, as a result of the COSY correlations between H-9/H-11 and H-11/H-12 and HMBC cross-peaks from H-9 to C-11 and from H_2-12 to C-11.

The relative configuration of **5** was determined through the NOESY correlations as well as a computer-generated lower energy conformation using MM2 force field calculations (S15, Supporting Information). NOESY correlations between H-1β (δ_H 2.39)/H-25, H-3/H-2β (δ_H 1.97), H-3/H_3-23, H-3/H_3-24, H-2β/H_3-23, H-5/H-9, H-9/H-12α (δ_H 1.38), H-9/H_3-27, H_3-23/H_3-25, H_3-25/H_3-26, H-13/H-12β (δ_H 1.80), and H-13/H_3-26 demonstrated a *trans-trans-trans* configuration for the A/B/C rings and the *R**-configuration of C-3. Furthermore, H-11 showed NOESY correlations with H-12β, H-13, H_3-25, and H_3-26, indicating the *R**-configuration at C-11. This was supported by the large coupling constant (J = 10.8 Hz) between H-11/H-9 and H-11/H-12α. Additionally, the large coupling constant ($J_{13,12\alpha}$ = 10.8 Hz) and the NOESY correlations between H-13 and H_3-26 implied a *trans* configuration at the C/D ring junction. Consequently, fatsicarpain E (**5**) was formulated as 3α,11α-dihydroxyolean-18(19)-en-28-oic acid.

The molecular formula of **7** was determined to be $C_{30}H_{46}O_4$ from the HRESIMS (m/z 469.3325 $[M - H]^-$), accounting for eight degrees of unsaturation. Comparison of the NMR data of **7** and **9** (Tables 1 and 3) showed that the structure of **7** should be very close to that of **9**, whose relative configuration was established by X-ray crystallographic analysis (Figure 3).[14] A methyl group in **9** was replaced by a hydroxymethyl group in **7** [δ_H 3.54 (1H, d, J = 11.4 Hz) and 3.43 (1H, d, J = 11.4 Hz); δ_C 71.2 (CH_2, C-23)], which was identified by the HMBC correlations from H_2-23 to C-3, C-4, and C-5 (S15, Supporting Information). The relative configuration of **7** assigned by the NOESY spectrum was compatible with that of **7** offered by computer modeling, in which the close contacts of atoms calculated in space were consistent with

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Table 2. ^{1}H NMR Spectroscopic Data (600 MHz, CD_3OD) for Compounds 2 and 5

	2	5
position	δ_H (J in Hz)	δ_H (J in Hz)
1	α: 1.08, m; β: 1.92, m	α: 1.46, m; β: 2.39, br d (14.4)
2	α: 1.79, m; β: 2.17, m	α: 1.45, m; β: 1.97, m
3	3.96, dd (12.0, 4.8)	3.30, br s
5	0.91, m	1.28, m
6	α: 1.63, m; β: 0.96, m	α: 1.42, m; β: 1.39, m
7	α: 1.36, m; β: 0.93, m	α: 1.50, m; β: 1.48, m
9	2.00, br s	1.54, d (10.8)
11	5.65, br d (10.2)	3.93, td (10.8, 4.2)
12	6.45, dd (10.2, 3.0)	α: 1.38, m; β: 1.80, dt (10.8, 4.2)
13		2.49, d (10.8)
15	α: 1.06, m; β: 1.72, m	α: 1.20, m; β: 1.58, m
16	α: 1.94, m; β: 1.70, m	α: 1.96, m; β: 1.57, m
19	α: 1.73, d (14.4); β: 2.53, d (14.4)	5.09, s
21	α: 1.38, d (9.6); β: 1.27, m	α: 1.48, m; β: 1.34, m
22	α: 1.39, d (9.6); β: 2.24, dd (9.6, 3.0)	α: 1.33, m; β: 2.16, br d (13.2)
23	0.83, s	0.91, s
24	1.04, s	0.86, s
25	0.96, s	1.10, s
26	0.81, s	1.04, s
27	0.99, s	0.85, s
29	0.94, s	0.99, s
30	0.81, s	0.97, s

the NOESY correlations. Thus, fatsicarpain G (7) was determined to be 3α-hydroxy-23-methoxyolean-11-en-13β,28-olide.

The leaves and twigs of *F. polycarpa* are abundant in 3α-hydroxy oleanane-type triterpenoids, but only **2** possesses a β-orientation of the 3-sulfate group. From a chemotaxonomic point of view, it is of interest to note that the 3*R**-configuration of these isolated triterpenoids is distinct from the 3*S**-configuration of triterpeniod saponins, which were obtained from the plant *F. japonica*.[2−9] However, the aglycone was not isolated, but the NMR evidence of the hydrolysis product proved the β-orientation of the 3-OH.

Anti-HBV effects of **1**−**9** were explored using the HepG2 2.2.15 (human hepatocellular carcinoma) cell model system. Compounds **1**, **3**, **4**, **6**, and **7** exhibited IC_{50} values of 18.9, 16.7, 28.8, 23.9, and 29.2 μM, respectively. The other compounds exhibited no discernible activity (IC_{50} > 50 μM). However, hepatitis B surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) in HepG2 2.2.15 cells were not significantly inhibited by the tested compounds.

This study was also aimed at evaluating the anti-*H. pylori* activity and the effects of these compounds on the suppression of human gastric epithelial AGS cells. Compounds **1**−**9** exhibited slight antibacterial activity against *H. pylori*, with minimum bactericidal concentrations of 64, 128, 64, 64, 128, 64, 64, 128, and >128 μg/mL, respectively. Compounds **1** and **9** exhibited moderate cytotoxicty against AGS cells, with IC_{50}'s of 10.1 and 9.0 μM, respectively. Compounds **4**, **5**, **7**, and **9** had IC_{50} values of 42.8, 47.6, 33.2, and 41.6 μM, respectively. The other compounds exhibited no discernible activity (IC_{50} > 50 μM).

The compounds were tested for antibacterial activity against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* in vitro. None of the compounds had statistically significant activity against *E. faecalis*, *E. coli*, *L. monocytogenes*, *P. aeruginosa*, or *S. enterica*. However, compound **3** had a minimum inhibitory concentration (MIC) value of 64 μg/mL against *S. aureus*. Compounds **1** and **3**−**6** showed specific antibacterial activity against *B. cereus*, with MIC values of 32, 32, 8, 64, and 2 μg/mL, respectively. Interestingly, compound **6** showed the highest anti-*Bacillus cereus* activity among the oleanane-type triterpenoids obtained from natural sources according to previous studies.[21,22]

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were determined with a JASCO P2000 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. The NMR spectra were recorded on a Varian Unity INOVA 600 FT-NMR spectrometer (600 MHz for ^{1}H; 150 MHz for ^{13}C, respectively). Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0 ppm) and CD_3OD (δ_H 3.30 and δ_C 49.0 ppm) as internal standard. High-resolution ESIMS spectra were obtained on a LTQ Orbitrap XL (Thermo Fisher Scientific) spectrometer. The crystallographic data were collected on a Bruker D8 Discover SSS X-ray diffractometer equipped with a closed molybdenum tube generator and parabolic Göbel mirror. Silica gel 60 (Merck, 230−400 mesh), LiChroprep RP-18 (Merck, 40−63 μm), and Sephadex LH-20 (Amersham Pharmacia Biotech.) were used for column chromatography (CC). Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for analytical thin-layer chromatography analyses. High-performance liquid chromatography (HPLC) was carried out using a Hitachi L-2130 pump equipped with a Hitachi L-2420 UV−vis detector at 220 nm and a semipreparative reversed-phase column (Merck, Hibar Purospher RP-18e, 5 μm, 250 × 10 mm).

Plant Material. Leaves and twigs of *Fatsia polycarpa* (7.1 kg) were collected at Hohuan mountain (2105 m elevation), Taiwan, in November 2009, and identified by one of the authors (C.-H.C.). A voucher specimen (FPL) was deposited in the Research Center for Biodiversity, China Medical University, Taiwan.

Extraction and Isolation. The air-dried and powdered leaves and twigs of *F. polycarpa* (7.1 kg) were extracted with MeOH for three days at room temperature (three times), and the combined extracts were concentrated in vacuo (under 35 °C). The resulting dark green gum was suspended in H_2O and extracted sequentially with CH_2Cl_2, EtOAc, and *n*-BuOH (saturated with H_2O). The CH_2Cl_2 extract (100 g) was subjected to CC on silica gel using *n*-hexane, *n*-hexane−EtOAc, and EtOAc−MeOH mixtures of increasing polarity for elution to furnish 40 fractions. Fraction 17 (2.86 g), eluted with *n*-hexane−EtOAc (1:4), was fractionated over Sephadex LH-20 (100% acetone) to afford **8** (108 mg). Fraction 20 (6.97 g), eluted with *n*-hexane−EtOAc (1:10), was chromatographed over silica gel, eluted in a step gradient manner with *n*-hexane−EtOAc−MeOH (10:1:0 to 0:0:100) to afford six subfractions. Subfraction 20-2 (3.16 g) was fractionated over Sephadex LH-20 (100% acetone) to yield a mixture (775 mg) that was subjected to RP-18 CC eluting with 65% MeOH in H_2O, 75% MeOH in H_2O, 85% MeOH in H_2O, 90% MeOH in H_2O, and 100% MeOH. Five fractions were obtained, of which fraction 4 (258 mg) was submitted to repeated CC over RP-18 using MeOH−H_2O−MeCN [20:60:20 + 0.2% FA (formic acid)] to give a mixture (74 mg) that was further separated by RP-18

Table 3. ^{13}C NMR Spectroscopic Data (150 MHz) for Compounds 1−7

	1[a]	2[b]	3[a]	4[a]	5[b]	6[a]	7[a]
C	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	32.5, CH_2	39.1, CH_2	31.9, CH_2	31.5, CH_2	36.8, CH_2	33.4, CH_2	32.6, CH_2
2	26.3, CH_2	25.2, CH_2	25.8, CH_2	25.8, CH_2	26.6, CH_2	25.3, CH_2	26.3, CH_2
3	77.0, CH	87.5, CH	73.0, CH	75.7, CH	76.7, CH	76.1, CH	76.8, CH
4	40.4, qC	39.7, qC	51.9, qC	37.7, qC	38.9, qC	37.4, qC	40.4, qC
5	42.5, CH	56.7, CH	43.4, CH	45.0, CH	50.3, CH	48.7, CH	42.4, CH
6	18.0, CH_2	19.4, CH_2	20.4, CH_2	18.1, CH_2	19.0, CH_2	18.2, CH_2	17.4, CH_2
7	31.9, CH_2	33.6, CH_2	31.8, CH_2	31.9, CH_2	36.7, CH_2	32.8, CH_2	30.7, CH_2
8	40.8, qC	41.9, qC	41.2, qC	42.4, qC	43.4, qC	42.8, qC	41.7, qC
9	54.2, CH	55.7, CH	53.9, CH	154.9, qC	57.0, CH	52.2, CH	53.0, CH
10	36.7, qC	37.7, qC	36.4, qC	38.9, qC	40.3, qC	38.2, qC	36.3, qC
11	127.2, CH	127.6, CH	126.5, CH	115.2, CH	71.5, CH	75.6, CH	135.8, CH
12	125.2, CH	126.5, CH	125.4, CH	120.7, CH	38.4, CH_2	121.8, CH	126.9, CH
13	137.1, qC	137.6, qC	136.8, qC	144.6, qC	41.6, CH	148.5, qC	89.9, qC
14	42.1, qC	43.2, qC	42.2, qC	40.8, qC	44.0, qC	41.7, qC	41.4, qC
15	24.8, CH_2	26.1, CH_2	24.8, CH_2	26.9, CH_2	30.6, CH_2	27.7, CH_2	25.3, CH_2
16	32.6, CH_2	34.0, CH_2	32.6, CH_2	23.6, CH_2	34.9, CH_2	22.7, CH_2	21.3, CH_2
17	48.1, qC	49.3, qC	48.0, qC	48.4, qC	50.1, qC	46.1, qC	44.0, qC
18	131.1, qC	133.6, qC	131.4, qC	39.5, CH	139.5, qC	40.4, CH	50.5, CH
19	40.4, CH_2	41.4, CH_2	40.5, CH_2	45.8, CH_2	132.6, CH	45.5, CH_2	37.3, CH_2
20	32.4, qC	33.4, qC	32.6, qC	30.6, qC	33.0, qC	30.6, qC	31.4, qC
21	36.8, CH_2	38.1, CH_2	36.8, CH_2	33.7, CH_2	34.9, CH_2	33.6, CH_2	34.3, CH_2
22	35.5, CH_2	36.8, CH_2	35.5, CH_2	32.1, CH_2	35.2, CH_2	32.2, CH_2	27.1, CH_2
23	71.3, CH_2	16.4, CH_3	209.7, CH	28.3, CH_2	29.5, CH_3	28.5, CH_2	71.2, CH_2
24	17.4, CH_3	28.5, CH_3	14.3, CH_3	22.4, CH_3	23.0, CH_3	22.3, CH_3	17.3, CH_3
25	18.0, CH_3	18.6, CH_3	17.9, CH_3	24.9, CH_3	17.3, CH_3	16.7, CH_3	18.0, CH_3
26	16.4, CH_3	17.2, CH_3	16.6, CH_3	20.2, CH_3	17.8, CH_3	18.8, CH_3	19.0, CH_3
27	19.9, CH_3	20.2, CH_3	19.9, CH_3	20.2, CH_3	15.3, CH_3	25.4, CH_3	18.4, CH_3
28	180.0, qC	180.9, qC	181.3, qC	183.8, qC	182.0, qC	184.0, qC	180.2, qC
29	32.2, CH_3	32.8, CH_3	32.2, CH_3	32.9, CH_3	30.9, CH_3	32.9, CH_3	33.3, CH_3
30	24.0, CH_3	24.5, CH_3	24.0, CH_3	23.5, CH_3	29.6, CH_3	23.5, CH_3	23.5, CH_3

[a] Spectra were measured in $CDCl_3$. [b] Spectra were measured in CD_3OD.

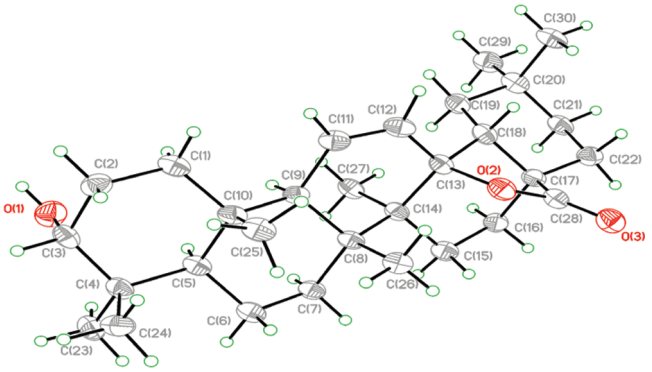

Figure 3. X-ray ORTEP diagram of compound **9**.

HPLC using H_2O−MeCN (35:65 + 0.3% FA) to isolate **4** (4 mg), **6** (15 mg), and **9** (40 mg). Subfraction 20-3 (1.96 g) was submitted to repeated silica gel CC using *n*-hexane−EtOAc mixtures of increasing polarity, to give 10 subfractions. Subfraction 20-3-6 (485 mg), eluted with *n*-hexane−EtOAc (2:1), was subjected to RP-18 CC using 75% MeOH in H_2O to yield a mixture (53 mg) that was further purified by RP-18 HPLC using MeOH−H_2O−MeCN (30:50:20 + 0.2% FA) to provide **3** (8 mg). Fraction 24 (1.5 g), eluted with EtOAc−MeOH (70:1), was subjected to silica gel CC (*n*-hexane−EtOAc−MeOH, from 10:1:0 to 0:10:1) to obtain 10 subfractions. Subfraction 24-6 (306 mg), eluted with *n*-hexane−EtOAc (1:3), was subsequently fractionated over Sephadex LH-20 (100% acetone) to yield a mixture (775 mg) that was further purified by RP-18 HPLC using MeOH−H_2O−MeCN (70:25:5) to give **1** (9 mg), **5** (2 mg), and **7** (2 mg). Fraction 30 (1.24 g), eluted with EtOAc−MeOH (1:1), was fractioned by RP-18 CC eluting with 65% MeOH in H_2O, 75% MeOH in H_2O, 85% MeOH in H_2O, 90% MeOH in H_2O, and 100% MeOH to afford 10 subfractions. Subfraction 30-1 (709 mg) was fractionated by RP-18 CC eluting with 60% MeOH in H_2O to provide a mixture (32 mg) that was separated by RP-18 HPLC using MeOH−H_2O−MeCN (50:45:5 + 0.2% FA) to provide **2** (2 mg).

Fatsicarpain A (***1***)*:* colorless needles; mp 271−273 °C; $[\alpha]^{25}_D$ −47 (*c* 0.9, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (4.24), 250 (4.03), 269 (3.95) nm; IR (KBr) ν_{max} 3325, 3032, 2958, 2923, 1710, 1654, 1616, 1446, 1375, 1200, 1112 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3; ESIMS *m*/*z* 469 $[M - H]^-$; HRESIMS *m*/*z* 469.3324 $[M - H]^-$ (calcd for $C_{30}H_{45}O_4$, 469.3312).

Fatsicarpain B (***2***)*:* white, amorphous powder; $[\alpha]^{25}_D$ +20 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 236 (4.18), 248 (3.98), 265

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(3.75) nm; IR (KBr) ν_{max} 3316, 3033, 2973, 2928, 1720, 1657, 1619, 1498, 1456, 1422, 1373, 1216, 1187, 1115 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; ESIMS m/z 533 $[M - H]^-$; HRESIMS m/z 533.2945 $[M - H]^-$ (calcd for $C_{30}H_{45}O_6S$, 533.2931).

*Fatsicarpain C (**3**):* white, amorphous powder; $[\alpha]^{25}_D$ −60 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (4.21), 249 (4.06), 268 (3.95) nm; IR (KBr) ν_{max} 3331, 3042, 2970, 2925, 2885, 1726, 1710, 1623, 1498, 1457, 1369, 1181, 1117 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; ESIMS m/z 467 $[M - H]^-$; HRESIMS m/z 467.3156 $[M - H]^-$ (calcd for $C_{30}H_{43}O_4$, 467.3156).

*Fatsicarpain D (**4**):* white, amorphous powder; $[\alpha]^{25}_D$ +71 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.44), 275 (3.95) nm; IR (KBr) ν_{max} 3328, 2958, 2923, 1712, 1654, 1616, 1446, 1378, 1150, 1114 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; ESIMS m/z 477 $[M + Na]^+$; HRESIMS m/z 477.3313 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3339).

*Fatsicarpain E (**5**):* white, amorphous powder; $[\alpha]^{25}_D$ −18 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3320, 2958, 2923, 1713, 1654, 1616, 1446, 1374, 1190, 1153 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; ESIMS m/z 495 $[M + Na]^+$; HRESIMS m/z 495.3437 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

*Fatsicarpain F (**6**):* white, amorphous powder; $[\alpha]^{25}_D$ +14 (*c* 1.1, $CHCl_3$); IR (KBr) ν_{max} 3235, 2958, 2923, 1712, 1647, 1625, 1446, 1385, 1185, 1150 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; ESIMS m/z 509 $[M + Na]^+$; HRESIMS m/z 509.3599 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_4Na$, 509.3601).

*Fatsicarpain G (**7**):* white, amorphous powder; $[\alpha]^{25}_D$ −10 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3450, 2958, 2923, 1745, 1656, 1627, 1446, 1375, 1200, 1157 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; ESIMS m/z 469 $[M - H]^-$; HRESIMS m/z 469.3325 $[M - H]^-$ (calcd for $C_{30}H_{45}O_4$, 469.3312).

Crystallographic Data and X-ray Structure Analysis of 1 (ref 14). A suitable colorless crystal of **1** was obtained by slow evaporation from CH_2Cl_2−MeOH (1:1) at room temperature. Diffraction intensity data were obtained on a Bruker D8 Discover SSS X-ray diffractometer equipped with a rotating Cu anode generator (wavelength λ = 1.54178 Å) and parabolic Göbel mirror yielding a quasi-parallel primary beam. Crystal data for **1**: $C_{30}H_{46}O_4$ (formula weight 470.67), approximate crystal size, 0.58 × 0.42 × 0.36 mm, orthorhombic, space group, $P2_12_12_1$, T = 110(2) K, a = 8.0495(3) Å, b = 17.7248(6) Å, c = 19.2463(7) Å, $\alpha = \beta = \gamma = 90°$, V = 2745.98(17) Å^3, D_c = 1.138 Mg/m^3, Z = 4, $F(000)$ = 1032, $\mu_{(MoK\alpha)}$ = 0.073 mm^{-1}. A total of 14 549 reflections were collected in the range 2.97° < θ < 29.32°, with 6412 independent reflections [$R_{(int)}$ = 0.0296]; completeness to θ_{max} was 99.8%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2; the number of data/restraints/parameters were 9412/0/331; goodness-of-fit on F^2 = 0.934; final R indices [$I > 2\sigma(I)$], R_1 = 0.0444, wR_2 = 0.1066; R indices (all data), R_1 = 0.0569, wR_2 = 0.1109, absolute structure parameter −0.2(10), largest difference peak and hole, 0.284 and −0.254 e/Å^3.

Crystallographic Data and X-ray Structure Analysis of 8 (ref 14). A suitable colorless crystal of **8** was grown by slow evaporation of the solution mixture CH_2Cl_2−MeOH (1:1) at room temperature. Mp: 259−261 °C (258−261 °C).[13] Diffraction intensity data were obtained on a Bruker D8 Discover SSS X-ray diffractometer equipped with a rotating Cu anode generator (wavelength λ = 1.54178 Å) and parabolic Göbel mirror, yielding a quasi-parallel primary beam. Crystal data for **8**: $C_{30}H_{46}O_3$ (formula weight 454.67), approximate crystal size, 0.62 × 0.50 × 0.43 mm, orthorhombic, space group, $P2_12_12_1$, T = 110(2) K, a = 11.1004(2) Å, b = 12.7518(3) Å, c = 20.9205(4) Å, $\alpha = \beta = \gamma = 90°$, V = 2961.30(10) Å^3, D_c = 1.020 Mg/m^3, Z = 4, $F(000)$ = 1000, $\mu_{(MoK\alpha)}$ = 0.064 mm^{-1}. A total of 10 319 reflections were collected in the range 3.12° < θ < 29.12°, with 6419 independent reflections [$R_{(int)}$ = 0.0196]; completeness to θ_{max} was 99.7%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2; the number of data/restraints/parameters were 6419/0/306; goodness-of-fit on F^2 = 1.009; final R indices [$I > 2\sigma(I)$], R_1 = 0.0519, wR_2 = 0.1487; R indices (all data), R_1 = 0.0616, wR_2 = 0.1534, absolute structure parameter −0.5(14), largest difference peak and hole, 0.421 and −0.340 e/Å^3.

Crystallographic Data and X-ray Structure Analysis of 9 (ref 14). A suitable colorless crystal of **9** was grown by slow evaporation of the solution mixture CH_2Cl_2−MeOH (1:1) at room temperature. Mp: 241−243 °C (240−244 °C).[13] Diffraction intensity data were recorded on a Bruker D8 Discover SSS X-ray diffractometer equipped with a rotating Cu anode generator (wavelength λ = 1.54178 Å) and parabolic Göbel mirror, yielding a quasi-parallel primary beam. Crystal data for **9**: $C_{30}H_{46}O_3$ (formula weight 454.67), approximate crystal size, 0.60 × 0.28 × 0.18 mm, triclinic, space group, $P1$, T = 110(2) K, a = 6.7903(6) Å, b = 7.0914(5) Å, c = 14.7228(13) Å, α = 78.885(7)°, β = 81.171(7)°, γ = 63.150(8)°, V = 618.86(9) Å^3, D_c = 1.220 Mg/m^3, Z = 1, $F(000)$ = 250, $\mu_{(MoK\alpha)}$ = 0.076 mm^{-1}. A total of 5421 reflections were collected in the range 2.83° < θ < 29.18°, with 4439 independent reflections [$R_{(int)}$ = 0.0412]; completeness to θ_{max} was 100.0%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2; the number of data/restraints/parameters were 4439/3/301; goodness-of-fit on F^2 = 0.921; final R indices [$I > 2\sigma(I)$], R_1= 0.0631, wR_2= 0.1479; R indices (all data), R_1 = 0.0849, wR_2 = 0.1561, absolute structure parameter −0.5(18), largest difference peak and hole, 0.361 and −0.306 e/Å^3.

Detection of the Sulfate Group. Compound **2** (1.0 mg) was refluxed with 10% HCl (1 mL) for 4 h and then extracted with EtOAc. An aliquot of the aqueous layer was treated with 70% $BaCl_2$ to give a white precipitate ($BaSO_4$).[19] In addition, the EtOAc extract was dried over anhydrous $MgSO_4$ and evaporated to yield a residue, which was subjected to a short silica gel column eluting with *n*-hexane−EtOAc (3:1) to give the corresponding hydrolysis product (0.6 mg). The ^1H NMR spectroscopic data of the product were in good agreement with those of oleanolic acid.[23]

Cytotoxicity Assay. Cytotoxicity was determined against HepG2 2.2.15 (human hepatocellular carcinoma cells) using the MTS assay (Promega, USA).[24] Briefly, HepG2 2.2.15 cells were treated with 2-fold serial dilutions of the tested compounds ranging from 1.25 to 40 μg/mL for 3 days, and the toxicity of cells was measured according to the manufacturer's protocol. All measurements were performed in triplicate, and the results were presented as relative percentage over that of the nontreated control group. The optimal nontoxic compound concentrations were then used in anti-HBV activity assays. Fluorouracil was used as the positive control. In addition, the AGS cell line (ATCC CRL 1739; human gastric adenocarcinoma) was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin). The in vitro cytotoxicity assay was carried out according to the procedures described previously.[25]

Anti-HBV Assay. The HepG2 2.2.15 cells established from a hepatoblastoma-derived cell line, HepG2, were kindly provided by Prof. Pei-Jer Chen (Hepatitis Research Center, National Taiwan University Hospital) and used as a platform for the anti-HBV assay.[26,27] This assay included measurement of the ability of each compound to inhibit the production of both hepatitis B surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg). Briefly, cells were cultured in modified Eagle's medium (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco, USA) without antibiotics at 37 °C in a humidified incubator with 5% CO_2. Confluent cultures were performed in 24-well tissue culture plates (Corning, USA), and the culture was treated with test compounds in culture medium with 2% fetal bovine serum every 3 days. The culture medium was removed at day 6, and the expression levels of HBsAg and HBeAg were detected by the enzyme immunoassay kit (Johnson and Johnson, USA) according to the manufacturer's instructions. The data of the control group, which was treated with

0.1% DMSO, for bioactivity assays were used to calculate the relative cell viability (%). Interferon-alpha (IFN-α) was used as the positive control.

Antibacterial Assay. *Helicobacter pylori* strain 26695 (ATCC 700392) was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and was grown on blood agar under microaerophilic conditions at 37 °C for 48−72 h. The antibacterial assay was conducted on the basis of previous reports.[25] Antibiotic assays were also carried out against seven pathogenic standard strains: *Bacillus cereus* (ATCC 9139), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC35150), *Listeria monocytogenes* (ATCC 7644), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13311), and *Staphylococcus aureus* (ATCC 29213). MICs were determined by the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute.[28] All bacteria strains were cultured on nutrient agar (Difco, USA) and incubated at 37 °C for 24 h. Bacterial inoculums were prepared in normal saline and diluted to a final density of 5×10^5 cfu/mL by comparison with a 0.5 McFarland turbidity standard. All compounds were dissolved in DMSO (Sigma, USA) and then in nutrient broth to reach a final concentration of 128 μg/mL. Serial 2-fold dilutions were made in a concentration range from 0.25 to 128 μg/mL. In each microtiter plate (Corning, USA), a column with a broad-spectrum antibiotic (ampicillin, Sigma, USA) was used as a positive control. All of the wells were examined by a spectrophotometer at 600 nm for bacterial growth. The MIC was defined as the lowest concentration at which no visible growth occurred in comparison with the positive control.

■ ASSOCIATED CONTENT

Supporting Information. Description of X-ray crystal data for **1**, **8**, and **9**, ^{1}H and ^{13}C NMR spectra for **1**−**7**, selected COSY and HMBC correlations for **5** and **7**, and selected NOESY correlations for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Plunkett, G. M.; Wen, J.; Lowry, P. P. *Plant Syst. Evol.* **2004**, *245*, 1–39.
(2) Aoki, T.; Tanio, Y.; Suga, T. *Phytochemistry* **1976**, *15*, 781–784.
(3) Aoki, T.; Suga, T. *Phytochemistry* **1978**, *17*, 771–773.
(4) Aoki, T.; Shido, K.; Takahashi, Y.; Suga, T. *Phytochemistry* **1981**, *20*, 1681–1686.
(5) Kitanaka, S.; Yasuda, I.; Kashiwada, Y.; Hu, C.-Q.; Bastow, K. F.; Bori, I. D.; Lee, K.-H. *J. Nat. Prod.* **1995**, *58*, 1647–1654.
(6) Sobolev, E. A.; Grishkovets, V. I.; Shashkov, A. S.; Tolkacheva, N. V.; Chirva, V. Y. *Chem. Nat. Compd.* **2000**, *36*, 538–539.
(7) Grishkovets, V. I.; Sobolev, E. A.; Shashkov, A. S.; Chirva, V. Y. *Chem. Nat. Compd.* **2000**, *36*, 166–169.
(8) Sobolev, E. A.; Grishkovets, V. I.; Kachala, V. V.; Shashkov, A. S.; Chirva, V. Y. *Chem. Nat. Compd.* **2001**, *37*, 292–293.
(9) Grishkovets, V. I.; Sobolev, E. A.; Kachala, V. V.; Shashkov, A. S.; Chirva, V. Y. *Chem. Nat. Compd.* **2002**, *38*, 264–267.
(10) Kemertelidze, É. P.; Kemoklidze, Z. S.; Dekanosidze, G. E.; Bereznyakova, A. I. *Pharm. Chem. J.* **2001**, *35*, 429–432.
(11) Lee, S. H.; Kim, S. Y.; Kim, D. W.; Jang, S. H.; Lim, S.-S.; Kwon, H. J.; Kang, T.-C.; Won, M. H.; Kang, I. J.; Lee, K. S.; Park, J.; Eum, W. S.; Choi, S. Y. *J. Microbiol. Biotechnol.* **2008**, *18*, 1613–1619.
(12) Hsieh, C. F. *Flora of Taiwan*, 2nd ed.; Editorial Committee of the Flora of Taiwan: Taipei, Taiwan, 1993; Vol. 3, pp 996−997.
(13) Caldwell, C. G.; Franzblau, S. G.; Suarez, E.; Timmermann, B. N. *J. Nat. Prod.* **2004**, *63*, 1611–1614.
(14) Crystallographic data for compounds **1**, **8**, and **9** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 813703, 812829, and 812830). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44−-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(15) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2010**, *27*, 79–132, and literature cited in previous reviews.
(16) Ganem, D.; Prince, A. M. *N. Engl. J. Med.* **2004**, *350*, 1118–1129.
(17) Egan, B. J.; Marzio, L.; O'Connor, H.; O'Morain, C. *Helicobacter* **2008**, *13*, 35–40.
(18) Ngassapa, O.; Beecher, C. W. W.; Pezzuto, J. M.; Farnsworth, N. R.; Henderson, T. O.; Boye, G. L. *J. Nat. Prod.* **1993**, *56*, 1872–1877.
(19) Perrone, A.; Masullo, M.; Bassarello, C.; Hamed, A. I.; Belisario, M. A.; Pizza, C.; Piacente, S. *J. Nat. Prod.* **2007**, *70*, 584–588.
(20) Cáceres-Castillo, D.; Mena-Rejón, G. J.; Cedillo-Rivera, R.; Quijano, L. *Phytochemistry* **2008**, *69*, 1057–1064.
(21) Liu, C. M.; Wang, H. X.; Wei, S. L.; Gao, K. *J. Nat. Prod.* **2008**, *71*, 789–792.
(22) Chen, J. J.; Fei, D. Q.; Chen, S. G.; Gao, K. *J. Nat. Prod.* **2008**, *71*, 547–550.
(23) Ikuta, A.; Itokawa, H. *Phytochemistry* **1988**, *27*, 2813–2815.
(24) O'Toole, S. A.; Sheppard, B. L.; McGuinness, E. P.; Gleeson, N. C.; Yoneda, M.; Bonnar, J. *Cancer Detect. Prev.* **2003**, *27*, 47–54.
(25) Hsu, Y.-M.; Weng, J.-R.; Huang, T.-J.; Lai, C.-H.; Su, C.-H.; Chou, C.-H. *Oncol. Rep.* **2010**, *23*, 1401–1405.
(26) Korba, B. E.; Milman, G. *Antivir. Res.* **1991**, *15*, 217–228.
(27) Korba, B. E.; Gerin, J. L. *Antivir. Res.* **1992**, *19*, 55–70.
(28) NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically M7-A5*; National Committee on Clinical Laboratory Standards, 2000; *20* (2).