

# Novel Cytotoxic Acylated Oligorhamnosides from *Mezzettia leptopoda*

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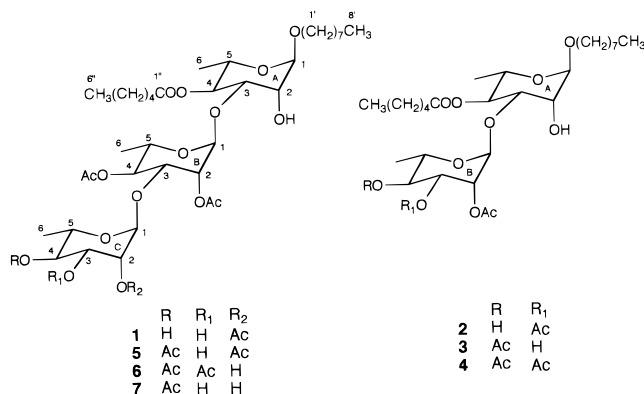
Activity-guided fractionation of a stem extract of *Mezzettia leptopoda* using human oral epidermoid carcinoma (KB) cells led to the isolation of seven highly acylated oligorhamnosides. Four of these constituents are novel, namely, *n*-octyl 2-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 8) (**1**); *n*-octyl 2,3-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 9) (**2**); *n*-octyl 2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 10) (**3**); and *n*-octyl 2,3,4-tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 11) (**4**). Three known compounds were identified as mezzettiasides 2 (**5**), 3 (**6**), and 4 (**7**), respectively, previously isolated from this same plant. The structures of novel compounds **1–4** were determined by spectroscopic methods. All the isolates were evaluated against a panel of human cancer cell lines in this study, and compounds **1–2** and **4–7** were found to be weakly cytotoxic toward KB and/or human colon and lung cancer cell lines.

*Mezzettia* Becc. is a small genus in the Annonaceae, indigenous to peninsular Malaysia and the island of Borneo.<sup>1</sup> In a continuing search for novel plant-derived anticancer agents, a CHCl<sub>3</sub>-soluble extract of the stems of *Mezzettia leptopoda* (Hook. f. & Thomas.) Oliver (Annonaceae) was investigated and found to be cytotoxic against the KB cell line (ED<sub>50</sub> 4.5  $\mu$ g/mL). Several classes of cytotoxic agents have been isolated previously from Annonaceae species, as exemplified by acetogenins,<sup>2–6</sup> aporphine alkaloids,<sup>7</sup> and clerodane<sup>8</sup> and labdane<sup>9</sup> diterpenoids.

Previously, six highly acylated oligorhamnosides, mezzettiasides 2–7, were obtained from *M. leptopoda*,<sup>10,11</sup> although no biological properties were reported for these compounds. In this paper, we discuss the isolation and structural characterization of four new acylated oligorhamnosides: *n*-octyl 2-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 8) (**1**); *n*-octyl 2,3-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 9) (**2**); *n*-octyl 2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 10) (**3**); and *n*-octyl 2,3,4-tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside 11) (**4**), along with three known compounds, mezzettiasides 2, 3, and 4 (**5–7**), from the stems of *M. leptopoda*. Compounds **1**, **2**, and **4–7** showed weak cytotoxic activity when evaluated against a panel of human cancer cell lines, while **3** was inactive in this regard.

## Results and Discussion

Compounds **5–7** were identified as the known compounds, mezzettiasides 2, 3 and 4, respectively, previously isolated from the stem bark of *M. leptopoda*, when the glycosyl units were identified by butanolysis followed by



trimethylsilylation and gas chromatography.<sup>10,11</sup> Acylated oligorhamnosides of this type have been reported from one other species in the Annonaceae, *Clesitopholis glauca*.<sup>12</sup>

The novel compound **1** was obtained as a colorless gum, and its molecular formula of C<sub>38</sub>H<sub>64</sub>O<sub>17</sub> was determined by HRFABMS, showing it to be an isomer of **7**. The IR spectrum of **1** showed a strong carbonyl (COOR) absorbance at 1732 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum of **1** (Table 1) exhibited chemical shifts and coupling constants very close to those of **7**,<sup>11</sup> including three anomeric protons at  $\delta$  4.71, 4.84, and 4.84 (each 1H, br s) and three acetyl groups at  $\delta$  2.12, 2.08, and 2.06 (each 3H, s). Analysis of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** indicated that there was an acetyl group at C-2 of the terminal rhamnose unit, from the observation of signals at  $\delta$  4.89 (1H, dd, *J* = 1.5, 3.2 Hz) and 74.1 (d), respectively. This inference was supported by selective INEPT, COSY, and HETCOR NMR experiments. Thus, in a COSY NMR experiment, cross peaks were observed between the signals of H-C1, H-C2, and H-C3 ( $\delta$  3.74, dd, *J* = 3.2, 9.6 Hz), confirming the presence of an acetyl substituent at the C-2 position of the terminal sugar moiety. In a selective INEPT NMR experiment performed on **1**, irradiation of H-B3 ( $\delta$  4.26, <sup>3</sup>*J*<sub>CH</sub> = 6 Hz) led to the enhancement of the carbon signals at  $\delta$  101.3 (d), 101.0 (d), and 68.0 (d), which were assignable to C-B1, C-C1, and C-B5, respectively. The <sup>1</sup>H and <sup>13</sup>C NMR

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**Table 1.**  $^1\text{H}$  NMR Spectral Data of Compounds 1–4 (300 MHz)

proton(s)	<b>1</b> <sup>a</sup>	<i>J</i> (Hz)	<b>2</b> <sup>b</sup>	<i>J</i> (Hz)	<b>3</b> <sup>b</sup>	<i>J</i> (Hz)	<b>4</b> <sup>b</sup>	<i>J</i> (Hz)
A-1	4.71 br s		4.93 br s		4.92 br s		4.93 br s	
A-2	3.92 <sup>c</sup>		4.23 <sup>c</sup>		4.23 <sup>c</sup>		4.23 <sup>c</sup>	
A-3	3.90 <sup>c</sup>		4.26 <sup>c</sup>		4.23 <sup>c</sup>		4.24 <sup>c</sup>	
A-4	5.16 t	9.6	5.54 t	9.9	5.50 t	9.9	5.55 t	9.6
A-5	3.78 dq	9.6, 6.2	3.97 dq	9.9, 6.2	3.98 dq	9.9, 6.2	3.96 dq	9.6, 6.2
A-6	1.15 d	6.2	1.32 d	6.2	1.32 d	6.2	1.33 d	6.2
B-1	4.84 br s		5.04 d	1.6	5.08 d	1.5	5.00 br s	
B-2	5.05 <sup>d</sup>		5.40 dd	3.5, 1.6	5.27 dd	3.6, 1.5	5.40 <sup>d</sup>	
B-3	4.26 dd	9.9, 3.4	5.56 dd	9.8, 3.5	4.44 dd	10.1, 3.6	5.67 dd	10.0, 3.3
B-4	5.04 t	9.9	3.85 t	9.8	5.31 dd	10.1, 9.8	5.41 t	10.0
B-5	4.07 dq	9.9, 6.2	4.26 <sup>c</sup>	9.8, 6.2	4.24 <sup>c</sup>	9.8, 6.2	4.39 dq	10.0, 6.2
B-6	1.16 d	6.2	1.52 d	6.2	1.27 d	6.2	1.25 d	6.2
C-1	4.84 br s							
C-2	4.89 dd	3.2, 1.5						
C-3	3.74 dd	9.6, 3.2						
C-4	3.39 t	9.6						
C-5	3.57 dq	9.6, 6.2						
C-6	1.26 d	6.2						
CH <sub>3</sub> CO	2.12 s		1.85 s		1.89 s		1.76 s	
	2.08 s		1.75 s		1.84 s		1.72 s	
	2.06 s						1.71 s	
1'	3.66 m		3.63 m		3.63 m			
2'–7', 3''–5''	1.15–1.68		1.22–1.70		1.23–1.70		1.23–1.71	
2''	2.52 m		2.52 m		2.61 m			
8', 6''	0.89 m		0.88 m		0.88 m		0.88 m	

<sup>a</sup> Measured in C<sub>6</sub>D<sub>6</sub>–CD<sub>3</sub>OD (1:6, v/v). <sup>b</sup> Measured in C<sub>6</sub>D<sub>6</sub>–CD<sub>3</sub>OD (4:1, v/v). <sup>c</sup> Overlapping signals. <sup>d</sup> Hidden signal.

signals of **1** and **7** were closely comparable, apart from the lack of the acetyl group at the C–C4 position in **7**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments for **1** (Tables 1 and 2) were made by selective INEPT,  $^1\text{H}$ – $^1\text{H}$  COSY, and  $^1\text{H}$ – $^{13}\text{C}$  HETCOR NMR experiments. Thus, the structure of **1** was established as *n*-octyl 2-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1→3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside, to which we have accorded the trivial name mezzettiaside **8**, consistent with the convention adopted for this type of compound by Waterman and colleagues.<sup>10,11</sup>

Compound **2**, having a molecular formula of C<sub>30</sub>H<sub>52</sub>O<sub>12</sub> as determined by HRFABMS, showed broad absorbances at 3530 (OH) and 1732 (COOR) cm<sup>−1</sup> in the IR spectrum. In the  $^1\text{H}$  NMR spectrum of this compound, only two anomeric proton signals were observed at  $\delta$  5.04 (1H, d, *J* = 1.6 Hz) and 4.93 (1H, br s), along with two acetyl groups at  $\delta$  1.85 and 1.75 (both 3H, s). This suggested that **2** contained one rhamnosyl unit less than compounds **1** and **5**–**7** and, hence, was a diacetyl dirhamnoside. The downfield chemical shifts of H–B2 and H–B3 observed at  $\delta$  5.40 (1H, dd, *J* = 1.6, 3.5 Hz) and 5.56 (1H, dd, *J* = 3.5, 9.8 Hz) suggested that two acetyl groups were located at the B2 and B3 positions, and this was supported by  $^{13}\text{C}$ , selective INEPT,  $^1\text{H}$ – $^1\text{H}$  COSY, and  $^1\text{H}$ – $^{13}\text{C}$  HETCOR NMR experiments. On the basis of the above evidence, the structure of **2** was therefore characterized as *n*-octyl 2,3-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1→3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside **9**).

Compound **3**, having the same molecular formula (C<sub>30</sub>H<sub>52</sub>O<sub>12</sub>) as that of **2** by its HRFABMS at *m/z* 605.3526 ([*M* + *H*]<sup>+</sup>, calcd 605.3537), showed very close IR,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectra to those of **2**. However, signals due to H–A4, H–B2, and H–B4 were shifted downfield to  $\delta$  5.50 (1H, t, *J* = 9.9 Hz), 5.27 (1H, dd, *J* = 1.5, 3.6 Hz), and 5.31 (1H, dd, *J* = 9.8, 10.1 Hz), respectively, indicating the presence of two acetyl units at the B2 and B4 positions. All of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments for **3** were made by selective INEPT,  $^1\text{H}$ – $^1\text{H}$  COSY, and  $^1\text{H}$ – $^{13}\text{C}$  HETCOR NMR experiments. Consequently, the structure of **3** was

determined as *n*-octyl 2,4-di-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1→3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside **10**).

The molecular formula of **4** was determined as C<sub>32</sub>H<sub>54</sub>O<sub>13</sub> by HRFABMS, one acetyl group more than those of **2** and **3**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **4** showed two anomeric protons [ $\delta$  4.93 (1H, br s) and 5.00 (1H, br s)] and carbon signals [ $\delta$  99.7 (d) and 100.5 (d)], respectively. In the  $^1\text{H}$  NMR spectrum of **4**, signals due to H–B2, H–B3, and H–B4 appeared downfield at  $\delta$  5.40 (1H, obscured), 5.67 (1H, dd, *J* = 3.3, 10.0 Hz), and 5.41 (1H, t, *J* = 10.0 Hz), suggesting the presence of three acetyl groups at the C–B2, C–B3, and C–B4 positions, which were confirmed by selective INEPT and  $^1\text{H}$ – $^1\text{H}$  COSY NMR experiments. Therefore, compound **4** was identified as *n*-octyl 2,3,4-tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl-(1→3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (mezzettiaside **11**).

Compounds **1**–**7** were evaluated against a panel of human cancer cell lines as summarized in Table 3. All compounds were found to be inactive against the human breast cancer (BC1), drug-resistant KB [assessed in the absence of vinblastine (KB–V1)], and the hormone-dependent human prostate cancer (LNCaP) cell lines in the panel. Compound **3** was inactive with all cell lines evaluated. The novel compound **2** showed weak cytotoxicity only against the Lu1 cell line (ED<sub>50</sub> 5.4  $\mu\text{g}/\text{mL}$ ), and **4** showed cytotoxic activity against the Lu1 cell line (ED<sub>50</sub> 6.1  $\mu\text{g}/\text{mL}$ ), and the KB–V1 cell line in the absence of vinblastine (ED<sub>50</sub> 5.6  $\mu\text{g}/\text{mL}$ ). Compounds **1** and **5**–**7** exhibited the most potent activity against the Col2 cell line, with ED<sub>50</sub> values of 8.2, 4.3, 4.9, and 6.2  $\mu\text{g}/\text{mL}$ , respectively. These test substances did not function by antimitotic mechanisms, as judged by the lack of activity observed with the rat glioma (ASK) test.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. The IR spectra were recorded on a Midac Collegian FT-IR interferometer;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at ambient temperature on either a Varian XL-300 or a Nicolet-360 instrument with tetramethylsilane (TMS) as internal

**Table 2.**  $^{13}\text{C}$  NMR Spectral Data of Compounds **1–4** (90.8 MHz)

carbon	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>b</sup>	<b>4</b> <sup>b</sup>
A-1	100.8 d <sup>c</sup>	99.9 d <sup>c</sup>	100.1 d <sup>c</sup>	99.7 d <sup>c</sup>
A-2	73.3 d	72.8 d	72.9 d	72.7 d
A-3	79.4 d	78.4 d	78.4 d	78.9 d
A-4	72.0 d	71.1 d	71.5 d	71.3 d
A-5	67.8 d	67.2 d	67.2 d	67.2 d
A-6	17.4 q	17.8 q	17.7 q	17.8 q
B-1	101.3 d	100.5 d	100.5 d	100.5 d
B-2	73.7 d	71.3 d	73.4 d	71.7 d
B-3	76.3 d	71.8 d	67.4 d	67.4 d
B-4	73.4 d	71.4 d	74.6 d	70.8 d
B-5	68.0 d	69.9 d	68.1 d	69.6 d
B-6	17.4 q	17.8 q	17.5 q	17.4 q
C-1	101.0 d			
C-2	74.1 d			
C-3	70.1 d			
C-4	73.8 d			
C-5	70.5 d			
C-6	17.2 q			
CH <sub>3</sub> CO	20.8 q	20.5 q	20.5 q	20.3 q
	21.0 q	20.3 q	20.3 q	20.2 q
	21.0 q			20.1 q
CH <sub>3</sub> CO	171.6 s	171.0 s	170.3 s	170.1 s
	172.1 s	170.2 s	171.2 s	170.2 s
	172.1 s			170.2 s
1'	67.9 t	68.1 t	68.1 t	68.1 t
2'-7' d	29.3 t	29.8 t	29.8 t	29.8 t
	26.1 t	26.5 t	26.5 t	26.5 t
	29.0 t	29.6 t	29.6 t	29.6 t
	29.1 t	29.7 t	29.7 t	29.7 t
	31.8 t	32.2 t	32.2 t	32.2 t
	22.7 t	23.0 t	23.0 t	23.0 t
8'	14.1 q	14.3 q	14.2 q	14.3 q
1''	173.1 s	173.6 s	174.0 s	173.5 s
2''	34.1 t	34.5 t	34.4 t	34.4 t
3''–5'' d	24.5 t	24.9 t	24.9 t	24.9 t
	31.3 t	31.6 t	31.6 t	31.6 t
	22.3 t	22.6 t	22.6 t	22.6 t
6''	13.9 q	14.0 q	14.0 q	14.0 q

<sup>a</sup> Measured in C<sub>6</sub>D<sub>6</sub>–CD<sub>3</sub>OD (1:6, v/v). <sup>b</sup> Measured in C<sub>6</sub>D<sub>6</sub>–CD<sub>3</sub>OD (4:1, v/v). <sup>c</sup> Multiplicities were established from HETCOR and DEPT spectra (s = singlet, d = doublet, t = triplet, and q = quartet). <sup>d</sup> Assignments of these signals are ambiguous due to the lack of resolution of the corresponding CH<sub>2</sub> signals in the  $^1\text{H}$  NMR spectrum.

**Table 3.** Cytotoxic Activity of Isolates Obtained from *M. leptopoda*<sup>a</sup>

com-pound	cell line <sup>b</sup>						
	BC1	Lu1	Co12	KB	KB-V1 <sup>+</sup>	KB-V1 <sup>-</sup>	LNCaP ASK
<b>1</b>	>20	19.7	8.2	>20	>20	>20	c
<b>2</b>	>20	5.4	>20	11.3	>20	>20	c
<b>3</b>	>20	>20	>20	>20	>20	>20	c
<b>4</b>	>20	6.1	9.0	12.7	5.6	>20	c
<b>5</b>	>20	8.6	4.3	6.2	>20	>20	c
<b>6</b>	>20	11.8	4.9	14.3	>20	>20	c
<b>7</b>	>20	19.4	6.2	15.4	>20	>20	c

<sup>a</sup> Results are expressed as ED<sub>50</sub> values (μg/mL). <sup>b</sup> Key: BC1 = human breast cancer; Lu1 = human lung cancer; Co12 = human colon cancer; KB = human oral epidermoid carcinoma; KB–V1<sup>+</sup> = drug-resistant KB assessed in the presence of vinblastine (1 μg/mL); KB–V1<sup>-</sup> = drug-resistant KB assessed in the absence of vinblastine; LNCaP = hormone-dependent human prostate cancer; ASK = rat glioma. <sup>c</sup> Inactive when tested at a concentration of 100 μg/mL.

standard. LRMS and HRMS were measured with a Finnigan MAT-90 instrument (70 eV). Column chromatography was carried out with Si gel G (Merck, 70–230 and 230–400 mesh).

**Plant Material.** The stems of *M. leptopoda* (Annonaceae) were collected in a tropical rain forest at Suratthani, Khao Thai Phat, Thailand, in May 1994, and identified by one of us (T. S.). Voucher specimens representing this collection have

been deposited at the Royal Forest Herbarium, Bangkok, Thailand, and the Field Museum of Natural History, Chicago, IL (A2571).

**Extraction and Isolation.** The air-dried stems (1.0 kg) of *M. leptopoda* were extracted with three changes of MeOH (1 × 3 L, 2 × 2 L). The resultant extracts were combined, concentrated under a vacuum, and then dissolved in 500 mL of MeOH and washed with hexanes (3 × 300 mL). The lower layer was then concentrated to dryness under reduced pressure and partitioned between 5% MeOH (300 mL) and CHCl<sub>3</sub> (3 × 300 mL). The CHCl<sub>3</sub>-soluble extract (32 g, ED<sub>50</sub> 4.5 μg/mL against the KB cell line) was subjected to Si gel column chromatography and eluted with hexane–Me<sub>2</sub>CO–MeOH mixtures (8:1:0.1–2:1:0.1) in a gradient (100 mL per fraction). The fourth fraction (1.4 g) [eluted with hexane–Me<sub>2</sub>CO–MeOH (5:1:0.1)] was absorbed on Si gel and further fractionation by column chromatography over Si gel using hexane–Me<sub>2</sub>CO–MeOH gradient mixtures. Subfractions 4–6, eluted with hexane–Me<sub>2</sub>CO–MeOH (7:1:0.1), were combined to afford **4** (8 mg). Subfractions 17–20, eluted with hexane–Me<sub>2</sub>CO–MeOH (6:1:0.1), were chromatographed over a Si gel column eluted with CHCl<sub>3</sub>–MeOH (50:1) to yield **2** (12 mg) and **3** (18 mg). The fifth fraction (2.2 g) [eluted from the first column with hexane–Me<sub>2</sub>CO–MeOH (3:1:0.1)] was subjected to Si gel column chromatography using CHCl<sub>3</sub>–MeOH mixtures (50:1–9:1) as solvents. Subfractions 2–3, eluted with CHCl<sub>3</sub>–MeOH (30:1), were combined and purified over a Si gel column using mixtures of CHCl<sub>3</sub>–Me<sub>2</sub>CO (10:1–3:1) to afford **5** (48 mg) and **6** (34 mg). Subfractions 6–8, eluted with CHCl<sub>3</sub>–MeOH (20:1), were purified by repeated Si gel column chromatography eluted with CHCl<sub>3</sub>–MeOH (40:1) to provide **1** (26 mg) and **7** (42 mg).

**Mezzettiaside 8 (1):** colorless gum; [α]<sub>D</sub> –66.4° (c 0.10 CHCl<sub>3</sub>); IR ν<sub>max</sub> (film) 3530, 2934, 1732, 1376, 1238 cm<sup>-1</sup>;  $^1\text{H}$  NMR data, see Table 1;  $^{13}\text{C}$  NMR data, see Table 2; positive FABMS (70 eV)  $m/z$  [M + H]<sup>+</sup> 793 (3), 691 (1), 663 (2), 605 (1), 419 (100), 375 (5), 311 (22); HRFABMS  $m/z$  793.2568 (calcd for C<sub>38</sub>H<sub>64</sub>O<sub>17</sub> + H, 793.2580).

**Mezzettiaside 9 (2):** colorless gum; [α]<sub>D</sub> –56.4° (c 0.10 CHCl<sub>3</sub>); IR ν<sub>max</sub> (film) 3530, 2938, 1732, 1376, 1238 cm<sup>-1</sup>;  $^1\text{H}$  NMR data, see Table 1;  $^{13}\text{C}$  NMR data, see Table 2; positive FABMS (70 eV)  $m/z$  [M + H]<sup>+</sup> 605 (1), 475 (3), 461 (1), 245 (6), 231 (66), 185 (23), 171 (100), 111 (10); HRFABMS  $m/z$  605.3528 (calcd for C<sub>30</sub>H<sub>52</sub>O<sub>12</sub> + H, 605.3537).

**Mezzettiaside 10 (3):** colorless gum; [α]<sub>D</sub> –54.4° (c 0.10 CHCl<sub>3</sub>); IR ν<sub>max</sub> (film) 3529, 2936, 1732, 1376, 1237 cm<sup>-1</sup>;  $^1\text{H}$  NMR data, see Table 1;  $^{13}\text{C}$  NMR data, see Table 2; positive FABMS (70 eV)  $m/z$  [M + H]<sup>+</sup> 605 (1), 475 (3), 461 (1), 245 (6), 231 (66), 185 (23), 171 (100), 111 (10); HRFABMS  $m/z$  605.3526 (calcd for C<sub>30</sub>H<sub>52</sub>O<sub>12</sub> + H, 605.3537).

**Mezzettiaside 11 (4):** colorless gum; [α]<sub>D</sub> –48.6° (c 0.10 CHCl<sub>3</sub>); IR ν<sub>max</sub> (film) 3531, 2936, 1734, 1380, 1240 cm<sup>-1</sup>;  $^1\text{H}$  NMR data, see Table 1;  $^{13}\text{C}$  NMR data, see Table 2; positive FABMS (70 eV)  $m/z$  [M + H]<sup>+</sup> 647 (2), 663 (2), 605 (1), 419 (100), 375 (5), 311 (22); HRFABMS  $m/z$  647.3653 (calcd for C<sub>32</sub>H<sub>54</sub>O<sub>13</sub> + H, 647.3643).

**Mezzettiaside 2 (5):** colorless gum; [α]<sub>D</sub> –32.5° (c 0.11, CHCl<sub>3</sub>); exhibited comparable spectral (IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FABMS) data to reported values.<sup>11</sup>

**Mezzettiaside 3 (6):** colorless gum; [α]<sub>D</sub> –47.6° (c 0.15, CHCl<sub>3</sub>); IR ν<sub>max</sub> (film) 3430, 2936, 1734, 1375, 1160 cm<sup>-1</sup>; exhibited comparable spectral ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FABMS) data to reported values.<sup>10</sup>

**Mezzettiaside 4 (7):** colorless gum; [α]<sub>D</sub> –56.4° (c 0.10, CHCl<sub>3</sub>); exhibited comparable spectral (IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FABMS) data to reported values.<sup>11</sup>

**Bioassay Evaluation Procedures.** Compounds **1–7** were evaluated for cytotoxic activity against a panel of human cancer cell lines according to established protocols.<sup>13</sup> Antimitotic activity was assessed using cultured rat glioma cells.<sup>14</sup> Results are given in Table 3.

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