Cytotoxic Monotetrahydrofuran Acetogenins from *Disepalum plagioneurum*

Véronique Éparvier,[†] Van Hung Nguyen,[‡] Odile Thoison,[†] Marie-Thérèse Martin,[†] Thierry Sévenet,[†] and Françoise Guéritte*[,†]

Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France, and Institute of Chemistry, VAST, Nghia Do, Cau Giay, Hanoi, Vietnam

Received April 14, 2006

The known plagionicin A (**1**) and eight new monotetrahydrofuran acetogenins, plagionicins B−D (**2**−**4**) and plagioneurins A−E (**5**−**9**), were isolated from the leaves of *Disepalum plagioneurum* by bioassay-guided purification. The structures of the new compounds were elucidated by spectroscopic methods. The new monotetrahydrofuran (mono-THF) acetogenins exhibited significant in vitro cytotoxicity against the KB cancer cell line, with IC_{50} values in the nanomolar range.

The Annonaceae is a large family of tropical plants that has been investigated intensively since the early 1980s after the discovery of the acetogenins.[1,2] These C_{35} or C_{37} fatty acid-derived natural products exhibit a broad biological activity spectrum including cytotoxicity in numerous tumor cell lines as well as antiparasitic, insecticidal, and immunosuppressive activities.[1,2]

In the course of our ongoing search to investigate bioactive plants from Vietnam, *Disepalum plagioneurum* (Diels) D.M. Johnson, belonging to the Annonaceae, was selected for a phytochemical study according to its potent cytotoxicity against KB cells. Thus, the EtOAc extract of the leaves produced 74% inhibition of cell growth at 0.1 $\mu g \cdot mL^{-1}$. Bioassay-directed fractionation of the EtOAc extract provided eight new acetogenins, named plagionicins B (**2**), C (**3**), and D (**4**) and plagioneurins A (**5**), B (**6**), C (**7**), D (**8**), and E (**9**). In contrast to plagionicins B−D (**2**−**4**), plagioneurins A−E (**5**−**9**) share in common the presence of an acetyl group on C-15 in their structure. We also report herein the isolation of the known plagionicin A (**1**), previously isolated from the seeds of *Polyalthia plagioneura* (syn. *D. plagioneurum*) and for which no configuration was described.[3] A literature survey revealed that only two other species of the genus *Disepalum*, *D. pulchrum*[4] and *D. anomalum*,[5] have been studied for their secondary metabolites. From *D. anomalum*, acetylated acetogenins have been isolated,[5] whereas alkaloids were found in *D. pulchrum*.[4] The structures of compounds **2** to **9** were identified as monotetrahydrofuran acetogenins by NMR spectroscopic and mass spectrometric analysis. The absolute configurations of the carbinol centers were assigned tentatively by NMR experiments using the Mosher esters method[6,7] and CD spectra. The new acetogenins (**2**−**9**) as well as plagionicin A (**1**) showed significant cytotoxicity against the KB cancer cell line.

Results and Discussion

D. plagioneurum was collected in Tam Kim, Cao Bang Province, in North Vietnam. The air-dried leaves were extracted successively with heptane, EtOAc, and MeOH. The EtOAc extract was found to be the most cytotoxic, producing 74% inhibition of KB cell growth at 0.1 $\mu g \cdot mL^{-1}$. Chromatographic treatment on silica gel followed by HPLC led to the known plagionicin A (**1**)[3] and the new acetogenins plagionicins B (**2**), C (**3**), and D (**4**) and plagioneurins A (**5**), B (**6**), C (**7**), D (**8**), and E (**9**).

Compound **1**, for which no configuration was determined previously, was identified as plagionicin A by comparison of its spectroscopic data to those of the described molecule.[3] According to Born[8] and Fujimoto[9] the methine signals resonating at δ 3.75 (2H, H-16 and H-19) and 3.32 ppm (H-15 and H-20) as well as the signals for carbons C-15 and C-20 at δ 74.5 and 74.6 and for carbons C-16 and C-19 at δ 83.0 indicated the presence of a mono-THF ring with two flanking hydroxyls in a *threo*/*threo* conformation (Tables 1 and 2). Moreover, the two THF methylene groups at δ 1.65 (H-17a and H-18a) and 1.95 (H-17b and H-18b) ($\Delta\delta \approx 0.3$) supported by two carbons at δ 28.4 revealed a *threo-trans-threo* configuration.[9−11] To determine the absolute configuration of **1**, the per-(*S*) and per-(*R*)-Mosher ester derivatives were prepared.[6,7] Their ^1H NMR chemical shifts were carefully assigned according to the $^1H-^1H$ COSY data (correlations between H-3/H-4, H-5/H-15, and H-16/H-20 and H-21). The sign of $\Delta\delta_{S-R}$ combined with the knowledge of the relative configuration of the THF ring[1] allowed the determination of the absolute configuration of the carbinol centers as 5*R*, 15*R*, and 20*R* (Tables 3 and 4). The absolute configuration at C-10 could not be solved by the Mosher ester method because precise assigments could not be made for protons H-9 and H-11. Finally, the CD spectrum was used to determine the lactone configuration.[12] The negative Cotton effect at 235 nm supported the conclusion that C-34 has the *S* configuration. Thus, plagionicin A (**1**) has the 5*R*, 15*R*, 16*R*, 19*R*, 20*R*, and 34*S* absolute configuration.

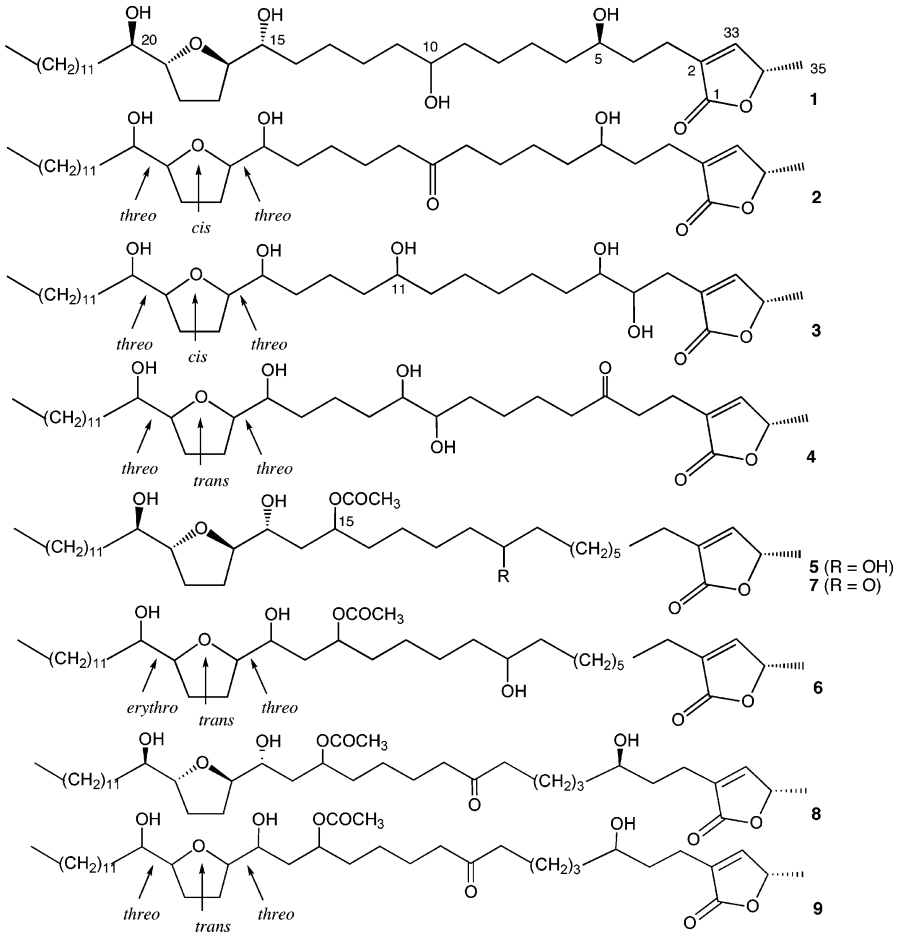
For all new compounds described above, the relative configurations of the THF ring with the two adjacent hydroxyl groups were determined in the same way as those of plagionicin A (**1**). The absolute configurations of the carbinol centers were then tentatively elucidated for compounds **5**, **7**, and **8** by the Mosher ester procedure.[13] Unfortunately, Mosher's ester derivatives could not be prepared from **2**, **3**, **4**, **6**, and **9**, due in part to problems concerning the purification of modified derivatives (data not shown).

Compound **2** and plagionicin A (**1**) showed the same basic spectroscopic features, except for the lack of a hydroxy group in **2**. In its HRESIMS, compound **2** showed a $[M + Na]^+$ peak at *m*/*z* 617.4391 corresponding to the molecular formula $C_{35}H_{62}O_7Na^+$. From the ^1H and ^{13}C NMR spectra (Tables 1 and 2), it could be deduced that compound **2** possesses an α,β-unsaturated γ-lactone unit and a mono-THF ring. In comparison to **1**, compound **2** showed the presence of a keto group with a carbonyl signal at δ 211.0 and one signal at δ 2.39 (4H) corresponding to the two adjacent methylene groups C-9 and C-11 (Tables 1 and 2). The carbonyl group was located at C-10 from the EIMS, which indicated a cleavage at C-10/C-11 with fragments at *m*/*z* 241 for **1** and *m*/*z* 239 for **2**, after the loss of two and one water molecules, respectively. The hydroxyl at C-5 was confirmed by the $^1H-^1H$ COSY spectrum, which showed a correlation of the C*H*-OH proton at δ 3.58 with H-3, H-4, and H-6. Similar to compound **1**, the mono-THF ring and its adjacent hydroxyls were all assigned as *threo* with signals at δ 3.40 (H-15 and H-20) and 3.79 ppm (H-16 and H-19) in the ^1H NMR spectrum and from ^{13}C NMR peaks at 74.1 (C-15), 82.6 (C-16), 82.7 (C-19), and 74.3 (C-20). However, the proton resonances of the two methylene groups at δ 1.70 (H-17a

* Corresponding author. Tel: 33 1 69 82 45 80. Fax: 33 1 69 07 72 47. E-mail: gueritte@icsn.cnrs-gif.fr.
[†] CNRS, Gif-sur-Yvette, France.
[‡] VAST, Hanoi, Vietnam.

10.1021/np060168j CCC: $33.50
Published on Web 09/08/2006

Chart 1



and H-18a) and 1.90 (H-17b and H-18b) ($\Delta\delta \approx 0.2$) supported by two ^{13}C NMR signals at δ 28.1 indicated a *cis* configuration. The *S* configuration at C-34 was determined by the CD experiment, which gave a negative Cotton effect at 235 nm. Compound **2** was named plagionicin B.

Compound **3** exhibited the molecular formula $C_{35}H_{64}O_8Na^+$, as deduced from its HRESIMS, which gave a $[M + Na]^+$ peak at *m*/*z* 635.4508. The IR absorption bands at 3400−3450 and 1746 cm^{-1} indicated the presence of hydroxy groups and an α,β-unsaturated γ-lactone moiety. The presence of this group was also given by the ^{1}H and ^{13}C NMR resonances at δ 7.20 (H-33), 5.20 (H-34), and 1.41 (H-35) (Table 1) and δ 171.9 (C-1), 152.2 (C-33), 130.7 (C-2), 78.2 (C-34), and 19.0 (C-35) (Table 2), respectively. The ^{1}H NMR spectrum showed seven signals between δ 3.38 and 3.77 corresponding to the methine protons of the dihydroxylated-THF group and to three other hydroxyl methine groups. Furthermore, the HMQC, HMBC, and ^{1}H−^{1}H COSY spectra, as well as the ^{13}C NMR spectroscopic data showing three signals at δ 71.4, 72.3, and 73.6, allowed the determination of the presence of the three hydroxy groups at C-11, C-4, and C-5, respectively (Tables 1 and 2). Similarly to compound **2**, the ^{13}C and ^{1}H NMR spectra of **3** indicated the presence of a *threo-cis-threo* mono-THF structure. The EIMS fragmentation confirmed the presence of the three hydroxy groups located at C-4, C-5, and C-11 by observing fragments at *m*/*z* 141 (cleavage C-4/C-5), 171 (cleavage C-5/C-6), and 271 (cleavage C-11/C-12). As for compounds **1** and **2**, the *S* configuration for C-34 of **3** was deduced from the CD spectrum, which gave a negative Cotton effect at 235 nm. Compound **3** was named plagionicin C.

The molecular formula of compound **4** was established as $C_{35}H_{62}O_8$ by the HRESIMS, which gave a $[M + Na]^+$ ion at *m*/*z* 633.4338. The presence of a α,β-unsaturated γ-lactone unit was provided by proton signals at δ 7.16 (H-33), 4.99 (H-34), and 1.41 (H-35) (Table 1) and by the ^{13}C NMR resonances at 173.4 (C-1), 150.5 (C-33), 132.4 (C-2), 77.5 (C-34), and 19.0 (C-35) (Table 2). A mono-THF ring and its vicinal hydroxyl groups were indicated by the carbon signals at δ 82.4 (C-16), 82.7 (C-19), 74.2 (C-15), and 74.3 (C-20). The ^{1}H NMR spectrum confirmed the presence of these functional groups with signals at δ 3.42 (H-15 and H-20), 3.81 (H-16 and H-19), 1.60 (2H), and 1.98 (C-17 and C-18). The EIMS of **4** provided evidence for the presence of four hydroxyls. The positions of the THF ring as well as the hydroxy and carbonyl groups were determined from the MS fragmentation data at *m*/*z* 153 (cleavage C-5/C-6), *m*/*z* 239 and 269 (cleavage C-10/C-11 and C-11/C-12), and *m*/*z* 341 (cleavage C-15/C-16). The relative configurations of the carbon centers C-15/16 and C-19/20 were found to be *threo* and the configuration of the THF ring *trans* by comparison of the chemical shifts with those of compound **1** of known configuration. Compound **4**, which possesses the *S* configuration at C-34 as deduced from its CD spectrum (negative Cotton effect at 235 nm), was named plagionicin D.

Compounds **5** to **9** showed mass spectrometric fragments corresponding to losses of *m*/*z* 60 and 18, indicating that they possess hydroxy and acetoxy groups. It is to be noted that the presence of the acetyl group at C-15 led to an upfield shift of C-17 at about 70 ppm in the ^{13}C NMR spectra. Consequently this signal could not be used alone to determine the *erythro* or *threo* relationship for C-17 and C-18. Compounds **5** and **6** gave the same $[M + Na]^+$ ion at *m*/*z* 689.4997, corresponding to the formula $C_{39}H_{70}O_8Na^+$. The LC-MS^2 and EIMS of these two compounds indicated the presence of three hydroxy and one acetyl group in each molecule. They gave an identical mass fragmentation pattern, which allowed the positioning of the di-OH-THF ring in each case, as well as those of the C-15 acetyl and C-10 hydroxy groups (Figure

Table 1. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Plagionicins A−D (**1**−**4**)

position	**1**	**2**	**3**	**4**
3	2.32, 2.40	2.35, 2.48	2.49 m	2.52, 2,44
4	1.6 m	1.20−1.58	3.64 m	2.73 m
5	3.50 m	3.58 m	3.40 m	
6	1.39 m	1.42 m	1.20−1.52	2.73 m
7, 8	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
9	1.16−1.50	2.39 m	1.20−1.52	1.20−1.52
10	3.50 m		1.35 m	3.42 m
11	1.16−1.50	2.39 m	3.56 m	3.42 m
12	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
13	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
14	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
15	3.32 m	3.40 m	3.38 m	3.42 m
16	3.75 m	3.79 m	3.77 m	3.81 m
17	1.65, 1.95	1.70, 1.90	1.70, 1.90	1.60, 1.98
18	1.65, 1.95	1.75, 1.95	1.70, 1.90	1.60, 1.98
19	3.75 m	3.79 m	3.77 m	3.81 m
20	3.32 m	3.40 m	3.38 m	3.42 m
21	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
22	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
23−29	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
30	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
31	1.16−1.50	1.20−1.58	1.20−1.52	1.20−1.52
32	0.80 t (7)	0.82 t (7)	0.82 t (7)	0.90 t (7)
33	7.03 d (1.5)	7.12 d (1.5)	7.20 d (1.5)	7.16 d (1.5)
34	4.99 dq (1.5, 6.5)	5.00 dq (1.5, 6.5)	5.20 dq (1.5, 6.5)	4.99 dq (1.5, 6.5)
35	1.37 d (6.5)	1.41 d (6.5)	1.41 d (6.5)	1.41 d (6.5)

Table 2. ^{13}C NMR Spectroscopic Data (150 MHz, $CDCl_3$) for Plagionicins A−D (**1**−**4**)

position	**1**	**2**	**3**	**4**
1	174.6	174.3	171.9	173.4
2	134.3	133.9	130.7	132.4
3	21.8	21.5−29.7	29.9	19.5
4	35.7	35.5	72.3	39.8
5	70.8	70.3	73.6	209.4
6	37.5	37.1	25.6−29.9	42.5
7, 8	23.0−29.9	21.4−29.6	25.6−29.9	23.1−25.2
9	37.5	42.6	23.7	33.4
10	71.8	211.0	37.2	73.9
11	37.7	42.7	71.4	73.9
12	30.0	25.2	37.1	33.4
13	26.0	25.3	25.5	25.5
14	34.2	33.8	34.1	28.8−31.9
15	74.5	74.1	74.2	74.2
16	83.0	82.6	82.7	82.4
17	28.4	28.1	28.1	28.9
18	28.4	28.1	28.1	28.8
19	83.0	82.7	82.7	82.7
20	74.6	74.3	74.3	74.3
21	34.4	34.1	33.8	28.8−31.9
22	23.0	25.7	25.6	28.8−31.9
23−29	23.0−30.0	21.5−29.7	25.6−29.9	28.8−31.9
30	32.2	21.5−29.7	31.9	31.9
31	23.0	21.5−29.7	22.6	19.5
32	14.4	14.7	14.1	14.1
33	150.0	149.7	152.3	150.5
34	78.0	77.7	78.2	77.5
35	19.4	19.1	19.0	19.0

1). The presence of the acetyl group was confirmed by the ^{1}H and ^{13}C NMR spectra, showing signals around δ 2.00 and 171 ppm (Tables 5 and 6). For compound **5**, the presence of the α,β-unsatured γ-lactone was provided by the ^{1}H NMR signals at δ 6.98 (H-35), 4.85 (H-36), and 1.28 (d, H-37) and the ^{13}C NMR peaks at δ 173.8 (C-1), 149.0 (C-35), 134.0 (C-2), 77.4 (C-36), and 19.2 (C-37). The mono-THF ring and its two flanking hydroxyls were indicated by carbon resonances at δ 82.2 (C-18 and C-21), 74.0 (C-22), 28.6 (C-20), and 28.6 (C-19) and proton signals at δ 3.77 (H-18), 3.76 (H-21), 3.33 (H-22), 3.32 (H-17), 1.62 (2H), and 1.90 (2H) (CH_2-19 and CH_2-20). These signals indicated a *threo-trans-threo* configuration. The correlations between protons of the THF ring, hydroxyl groups, and lactone moiety were established on the basis of the ^{1}H−^{1}H COSY spectrum. The (*R*)- and (*S*)-Mosher ester derivatives were then prepared to determine the configuration of **5**. The *R* configuration could thus be assigned to C-17, C-18, C-21, and C-22, according to the shifts of the proton signals as shown in Table 7. Finally, the configuration at C-36 of the lactone moiety was determined as *S* from the negative Cotton effect at 235 nm. Compound **5** was named plagioneurin A.

Table 3. ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters for the Determination of the Absolute Configuration at C-5 for **1** and **8**

	proton			
	3	4	5	6
1 *S*-MTPA	2.20	1.86	5.06	1.65
1 *R*-MTPA	2.28	1.89	5.02	1.52
$\Delta\delta_{S-R}$	−0.08	−0.03	+0.04	+0.13
configuration			*R*	
8 *S*-MTPA	2.33	1.70	5.08	1.62
8 *R*-MTPA	2.35	1.90	5.08	1.60
$\Delta\delta_{S-R}$	−0.02	−0.20		+0.02
configuration			*R*	

Table 4. ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters for the Determination of the Absolute Configuration at C-15 and C-20 of **1**

	proton					
	14	15	16	19	20	21
1 *S*-MTPA	1.62	5.05	3.88	4.06	4.90	1.59
1 *R*-MTPA	1.53	5.04	3.90	4.08	4.09	1.49
$\Delta\delta_{S-R}$	+ 0.09		−0.02	−0.02		+0.10
configuration		*R*			*R*	

As mentioned earlier, the EIMS of compound **6** (Figure 1) was identical to that of **5**, indicating that these two compounds are isomers. The ^{1}H and ^{13}C NMR data of **6** (Tables 5 and 6) confirmed the position of the C-10 hydroxy and C-15 acetoxy groups as well as that of the mono-THF ring with its two adjacent hydroxyls. On the basis of the chemical shifts of H-22 at δ 3.80, H-19 and H-20 at 1.68 and 1.92 ppm, and C-22, C-20, and C-19 respectively at δ 71.5, 25.1, and 29.1, an *erythro-cis-threo* configuration was attributed to the mono-THF ring system. From the CD spectrum, the 36*S* absolute configuration could be established. Compound **6** was named plagioneurin B.

The molecular formula of **7** was established as $C_{39}H_{68}O_8$ by MALDITOFMS, which gave a peak at *m*/*z* 687.4826 for the $[M + Na]^+$ ion. In comparison to **5** and **6**, the EIMS fragmentation (Figure 1) indicated the presence of a keto group instead of a hydroxyl at C-10. This was also confirmed by analysis of the 1D and 2D (COSY and HMBC) NMR spectra. The mono-THF ring with its two flanking hydroxyls was identified by ^{13}C NMR signals at δ 70.1 (C-17), 82.2 (C-18), 28.6 (C-19 and C-20), 82.9 (C-21), and 74.0 (C-22) as well as proton signals at δ 1.65 and 1.95 ($\Delta\delta = 3$) for the two methylene groups and at δ 3.38, 3.79, 3.80, and 3.39 for protons at the 17, 18, 21, and 22 positions (Tables 5 and 6). These chemical shifts indicated a *threo-trans-threo* configuration for the mono-THF ring system. The (*R*)- and (*S*)-Mosher ester derivatives of **7** were prepared to determine its absolute configuration. Examination of the NMR data allowed the absolute configuration to be demonstrated as *R* for C-17 and C-22 (Table 7). Knowing the relative configuration of the THF ring, it was thus concluded that compound **7** has the 17*R*, 18*R*, 21*R*, and 22*R* absolute configuration. Moreover, the *S* configuration at C-36 was deduced from the CD spectra (negative Cotton effect at 235 nm) of **7**, which was named plagioneurin C.

The HRESIMS of compounds **8** and **9** displayed an identical $[M + Na]^+$ ion at *m*/*z* 703.4805 and 703.4813, respectively, corresponding to the molecular formula $C_{39}H_{68}O_9Na$. The LC-MS

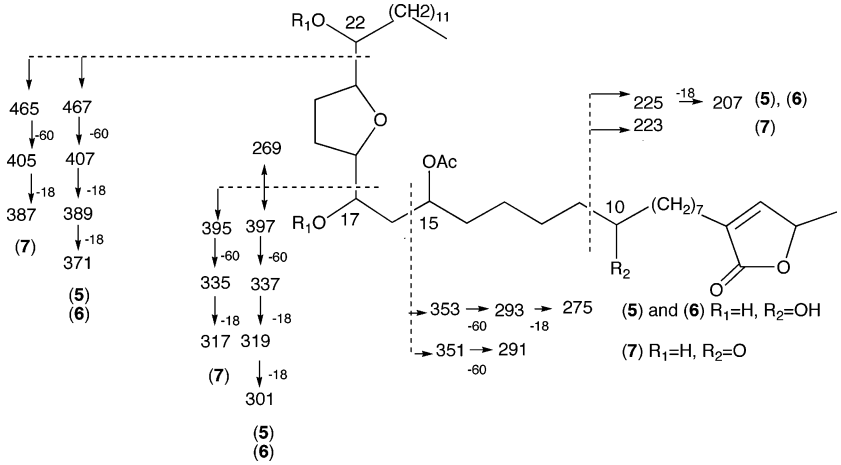


Figure 1. Significant EIMS fragment ion *m*/*z* values of **5**−**7**.

Table 5. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds **5**−**9**

position	**5**	**6**	**7**	**8**	**9**
3	2.12 dq (1, 8)	2.24 dq (1, 9)	2.22 t (7)	2,35, 2,42	2.37, 2,43
4	1.18−1.62	1.18−2.00	1.18−1.65	1.41 m	1.41 m
5	1.18−1.62	1.18−2.00	1.18−1.65	3.55 m	3.55 m
6	1.18−1.62	1.18−2.00	1.18−1.65	1.58 m	1.58 m
7	1.18−1.62	1.18−2.00	1.18−1.65	1.35	1.35 m
8	1.20 m	1.37 m	1.52 m	1.15−1.57	1.15−1.58
9	1.45 m	1.40 m	2.35 ddd (2, 7, 12)	2.36 m	2.36 m
10	3.39 m	3.55 m			
11	1.45 m	1.40 m	2.35 ddd (2, 7, 12)	2.36 m	2.36 m
12	1.18−1.62	1.18−2.00	1.18−1.65	1.15−1.57	1.15−1.58
13	1.18−1.62	1.18−2.00	1.18−1.65	1.15−1.57	1.15−1.58
14	1.57 m	1.57 m	1.56 m	1.53 m	1.53 m
15	5.05 m	5.10 m	5.08 m	5.06 m	5.07 m
16	1.59 m	1.58 m	1.60 m	1.58 m	1.58 m
17	3.32 m	3.37 m	3.38 m	3.40 m	3.40 m
18	3.77 m	3.76 m	3.79 m	3.73 m	3.73 m
19	1.62, 1.90	1.65, 1.95	1.65, 1.95	1.64, 1.94	1.65, 1.94
20	1.62, 1.90	1.65, 1.95	1.65, 1.95	1.64, 1.94	1.65, 1.94
21	3.76 m	3.82 m	3.80 m	3.75 m	3.75 m
22	3.33 m	3.80 m	3.39 m	3.39 m	3.39 m
23	1.46 m	1.35 m	1.38	1.47 m	1.47 m
24	1.20 m	1.18−2.00	1.18−1.65	1.15−1.94	1.15−1.94
25−31	1.18−1.62	1.18−2.00	1.18−1.65	1.15−1.94	1.15−1.94
32	1.26 m	1.22 m	1.39 m	1.21 m	1.21 m
33	1.17 m	1.26 m	1.28 m	1.26 m	1.26 m
34	0.76 t (6)	0.84 t (7)	0.83 t (6)	0.87 t (7)	0.88 t (7)
35	6.98 d (1.5)	6.95 d (1.5)	6.95 d (1.5)	6.80 d (1.5)	6.80 d (1.5)
36	4.85 dq (1.5, 6.5)	4.97 dq (1.5, 6.5)	4.95 dq (1.5, 6.5)	4.85 dq (1.5, 6.5)	4.85 dq (15, 6.5)
37	1.38 d (6.5)	1.39 d (6.5)	1.38 d (6.5)	1.39 d (6.5)	1.39 d (6.5)
CH_3−C═O	1.99 s	2.05 s	2.21 s	2.02 s	2.02 s

and NMR spectroscopic data indicated the presence of three hydroxyls, one carbonyl, and one acetate group, as well as an α,*β*-unsatured *γ*-lactone and a mono-THF ring. Moreover, the EI mass fragmentation pattern of these two compounds as well as their NMR spectra were almost superimposable. From the EIMS data, fragment ions at *m*/*z* 367, 239, and 155 indicated the positions of the acetate, keto, and hydroxy groups at positions 15, 10, and 5, respectively. For compound **8**, the proton signals at *δ* 3.73 and 3.75 (H-18 and H-21), 3.39 and 3.40 (H-22 and H-17), 1.64 (H-19a and H-20a), and 1.94 (H-19b and H-20b) as well as the carbon signals at *δ* 70.1 (C-17), 74.1 (C-22), and 29.3 (C-19 and C-20) established a *threo-trans-threo* configuration for the di-OH THF ring (Tables 5 and 6). The configuration at C-5 (*R*), C-17 (*R*), and C-22 (*R*) could be determined from the ^{1}H NMR spectroscopic data of the (*S*)- and (*R*)-MTPA esters of **8** (Tables 3 and 7). This indicated also an *R* configuration for carbons 18 and 21, and the 36*S* configuration was given by the negative Cotton effect at 235 nm. Compound **8** was named plagioneurin D.

On the basis of the NMR (Tables 5 and 6) and MS data, it was deduced that compound **9** is a stereoisomer of **8**. The relative configuration of the mono-THF ring with its two adjacent hydroxy groups was determined as *threo-trans-threo* on the basis of the comparison of the ^{1}H and ^{13}C NMR data with those of compound **8**. The absolute configuration of the lactone moiety of **9** was determined as *S* by the CD experiment. Compound **9** was named plagioneurin E.

Compounds **1**−**9** were subjected to a cytotoxicity assay against the KB cancer cell line (Table 8). Most of the acetogenins showed significant cytotoxicity. Plagionicin B (**2**) had moderate activity, with an IC_{50} value of 1.4×10^{-6} M, whereas compounds **1**, **3**, and the acetylated acetogenins **5**−**9** showed cytotoxicity in the nanomolar range.

Experimental Section

General Experimental Procedures. Optical rotations were measured at 20 °C on a JASCO P1010 polarimeter, and CD spectra were recorded on a Jobin Yvon CD6 dichrograph. UV spectra were recorded in MeOH on a Varian Cary 100 spectrophotometer and IR spectra on a Perkin-Elmer Spectrum BX FT-IR spectrometer. The NMR spectra were recorded on an Aspect AMX 600 spectrometer. Chemical shifts

Table 6. ^{13}C NMR Spectroscopic Data (150 MHz, $CDCl_3$) for Compounds **5**−**9**

position	**5**	**6**	**7**	**8**	**9**
1	173.8	173.9	174.0	173.8	174.2
2	134.0	134.3	134.1	134.2	133.9
3	25.1	25.4	22.6−29.0	21.4	21.4
4	26.0−29.7	25.6−29.7	22.6−29.0	35.4	35.5
5	26.0−29.7	25.6−29.7	22.6−29.0	70.3	70.3
6	26.0−29.7	25.6−29.7	22.6−29.0	37.1	37.1
7	26.0−29.7	25.6−29.7	22.6−29.0	25.2	25.6
8	25.4	25.4	23.5	23.4	23.6
9	37.1	37.5	42.5	42.4	42.5
10	71.4	71.7	211.0	211.0	211.2
11	37.3	37.3	42.7	42.6	42.6
12	22.3	25.0	23.7	28.6-31.9	28.2−29.0
13	25.3	25.6−29.7	24.9	24.9	25.0
14	34.4	35.0	34.7	34.6	34.6
15	71.3	71.4	71.3	71.3	71.6
16	38.4	38.6	38.6	37.1	37.1
17	70.1	70.4	70.1	70.1	70.3
18	82.2	82.2	82.2	82.2	82.3
19	28.6	29.1	28.6	29.3	29.3
20	28.6	25.1	28.6	29.3	29.3
21	82.8	82.8	82.9	82.9	82.6
22	74.0	71.5	74.0	74.1	74.2
23	33.3	32.5	33.4	33.5	33.5
24	25.6	25.6−29.7	25.5	28.6−31.9	28.5−31.7
25−31	26.0−28.7	25.6−29.7	22.6−29.0	28.6−31.9	28.5−31.7
32	30.3	31.9	31.9	31.8	31.7
33	22.5	22.7	22.6	22.6	22.7
34	14.1	14.1	14.1	14.1	14.1
35	149.0	148.9	148.9	149.0	149.0
36	77.4	77.4	77.4	77.3	77.7
37	19.2	19.2	19.2	19.2	19.1
$CH_3-C{=}O$	171.3	171.3	171.4	171.0	172.0
$CH_3-C{=}O$	21.2	21.2	21.2	21.2	21.2

Table 7. ^1H NMR Data of the (*S*)- and (*R*)-Mosher Esters of **5**, **7**, and **8**

	proton					
	16	17	18	21	22	23
5 *S*-MTPA	1.81	5.09	3.91	3.94	4.97	1.58
5 *R*-MTPA	1.79	5.17	4.00	4.04	5.03	1.51
$\Delta\delta_{S-R}$	+0.02		−0.09	−0.10		+0.07
configuration		*R*			*R*	
7 *S*-MTPA	1.80	5.10	3.88	3.92	4.97	1.56
7 *R*-MTPA	1.78	5.16	3.98	4.02	5.02	1.52
$\Delta\delta_{S-R}$	+0.02	−0.06	−0.10	−0.10	−0.05	+0.04
configuration		*R*			*R*	
8 *S*-MTPA	1.82	5.10	3.89	3.93	4.97	1.57
8 *R*-MTPA	1.78	5.16	3.99	4.02	5.03	1.52
$\Delta\delta_{S-R}$	+0.04	−0.06	−0.10	−0.09	−0.06	+0.05
configuration		*R*			*R*	

Table 8. In Vitro Cytotoxicity of Compounds **1**−**9**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	docetaxel[a]
KB cell line [IC_{50} (nM)]	2.0	1400	6.0	26	2.4	6.5	26	2.8	2.6	0.2

[a] Docetaxel was used as the standard positive control

(relative to TMS) are in ppm, and coupling constants (in brackets) in Hz. Mass spectra were obtained for EI on an AutoMass Multi from Thermo-Finnigan, for ESI on a LCT from Micromass or Thermo-Finnigan Navigator, and for MALDITOF on a Voyager-DE STR. Measurements of exact mass were obtained using a ZAB-SEQ mass spectrometer. Column chromatography was performed using Merck H60 silica gel. HPLC was performed on a Waters Alliance 2690 with a Waters 600^E system controller. Reversed-phase Themohypersil Kromasil C_{18} 5 μm (250 × 10 mm) and reversed-phase Nova-Pak Hypercarb C_{18} 5 μm (150 × 3.9 mm) columns were used for preparative and analytical purposes.

Plant Material. The leaves of *Disepalum plagioneurum* were collected in June 2000 at Tam Kim, Cao Bang Province, in North Vietnam by Dr. A. Gramain, under the reference VN 682, and identified by Dr. N. Binh (Institute of Ecology), VAST, Hanoï, Vietnam; a herbarium specimen has been deposited at the Institute of Ecology (VAST, Hanoï) and at the Laboratoire de Phanerogamie, Museum National d'Histoire Naturelle, Paris, France.

Extraction and Isolation. The air-dried leaves (2 kg) of *D. plagioneurum* were extracted successively by heptane, EtOAc, and MeOH at room temperature. One part of the EtOAc extract (45 g) was separated into 16 fractions by column chromatography on silica gel with a gradient of *n*-hexane−CH_2Cl_2 (50:50 to 0:100) and CH_2Cl_2−MeOH (100:0 to 80:20). Plagionicin A (**1**) (3.7 g) was obtained directly from fraction 12. All other fractions were subjected to the cytotoxic assay, showing fractions 2, 4, 7, 11, and 15 to be the most active. Plagionicin B (**2**) (10.5 mg) was purified from fraction 11 by HPLC (reversed-phase Themohypersil Kromasil C_{18} 5 μm column, 250 × 10 mm, flow rate 3 mL/min, MeCN−H_2O (70:30 to 100:0), and then by a reversed-phase Nova-Pak Hypercarb C_{18} 5 μm column, 150 × 3.9 mm, flow rate 1 mL/min, AcOEt−CH_2Cl_2 (50:50)). The same procedure was used to purify fraction 15, which gave plagionicin C (**3**) (18.6 mg) and plagionicin D (**4**) (9.9 mg). Fractions 2, 4, 7, 11, and 15 were purified by HPLC (Themohypersil Kromasil C_{18} 5 μm column, 250 × 10 mm, flow rate 3 mL/min, MeCN−H_2O (70:30 to 100:0)). Fraction 4 gave plagioneurin A (**5**) (66.5 mg) and plagioneurin B (**6**) (8.7 mg), whereas fraction 2 gave plagioneurin C (**7**) (32.3 mg) and fraction 7, plagioneurin D (**8**) (33.9 mg) and plagioneurin E (**9**) (13.8 mg).

Plagionicin A (1): white, waxy solid; $[\alpha]^{24}_D$ +12.7 (*c* 1.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (3.67) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−1.0), 219 (5.1), 210 (3.2); IR (KBr) ν_{max} 3411, 2920, and 2850, 1747, 1464, 1319 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; EIMS *m*/*z* 578 [M − H_2O], 560 [M − 2 H_2O], 542 [M − 3 H_2O], 524, 397, 379, 361, 343, 327, 325, 309, 291, 273, 269, 241, 223, 205, 155, 137; HRESIMS *m*/*z* $[M + Na]^+$ 619.4543 (calcd for $C_{35}H_{62}O_7Na$, 619.4550).

Plagionicin B (2): white, waxy solid; $[\alpha]^{23}_D$ +4.4 (*c* 1.8, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 227 (3.15) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−1.9), 216 (3.1), 204 (3.7); IR (KBr) ν_{max} 3448, 2919, 2850, 1740, 1469 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; EIMS *m*/*z* 594 [M], 576 [M − H_2O],

558 [M − $2H_2O$], 540 [M − $3H_2O$], 377, 325, 307, 289, 239, 221; HRESIMS *m/z* [M + Na]$^+$ 617.4391 (calcd for $C_{35}H_{64}O_7Na$, 617.4393).

Plagionicin C (3): white, waxy solid; $[\alpha]^{23}_D$ +22 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 227 (3.13) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−0.9), 213 (4.1), 209 (4.8); IR (KBr) ν_{max} 3408, 2961, 2917, 2850, 1746, 1462, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; EIMS *m/z* 594 [M − H_2O], 576 [M − $2H_2O$], 558 [M − $3H_2O$], 395, 377, 359, 341, 325, 307, 289, 271, 269, 253, 235, 171, 153, 141; HRESIMS *m/z* [M + Na]$^+$ (635.4508 calcd for $C_{35}H_{64}O_8Na$, 635.4499).

Plagionicin D (4): white, waxy solid (9.9 mg); $[\alpha]^{23}_D$ +22 (*c* 0.4, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 227.00 (3.16) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−1.9), 219 (1.9), 216 (2.3); IR (KBr) ν_{max} 3408, 2961, 2919, 2845, 1754, 1702, 1462, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; EISM *m/z* 592 [M − H_2O], 574 [M − $2H_2O$], 556 [M − $3H_2O$], 393, 375, 357, 341, 323, 205, 287, 269, 251, 139, 233, 221, 203, 153; HRESIMS *m/z* [M + Na]$^+$ 633.4338 (calcd for $C_{35}H_{62}O_8$, 633.4342).

Plagioneurin A (5): yellow oil (66.5 mg); $[\alpha]^{24}_D$ +30.3 (*c* 0.8, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 218 (3.70) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) (−1.0), 218 (3.5), 209 (4.0); IR (KBr) ν_{max} 3446, 2925, 2855, 1736, 1734, 1457, 1246 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 5; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 6; EIMS *m/z* 606 [M − $HOCOCH_3$], 588 [M − $HOCOCH_3$ − H_2O], 570 [M − $HOCOCH_3$ − $2H_2O$], 552 [M − $HOCOCH_3$ − $3H_2O$], 407, 397, 389, 371, 353, 337, 319, 301, 293, 275, 269, 225, 207, 111; HRMALDITOFMS *m/z* [M + Na]$^+$ 689.4997 (calcd $C_{39}H_{70}O_8Na$, 689.4968).

Plagioneurin B (6): yellow oil (8.7 mg); $[\alpha]^{24}_D$ +23.4 (*c* 0.1, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 212 (3.90) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−0.4), 218 (2.1), 209 (2.2); IR (KBr) ν_{max} 3437 (OH), 2924, 2853 (CH), 1740 (OC═O), 1465 (CH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 5; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 6; EIMS *m/z* 606 [M − $HOCOCH_3$], 588 [M − $HOCOCH_3$ − H_2O], 570 [M − $HOCOCH_3$ − $2H_2O$], 552 [M − $HOCOCH_3$ − $3H_2O$], 467, 407, 397, 389, 371, 353, 337, 319, 301, 293, 275, 269, 225, 207, 111; HRMALDITOFMS *m/z* [M + Na]$^+$ 689.4997 (calcd for $C_{39}H_{70}O_8Na$, 689.4968).

Plagioneurin C (7): yellow oil (32.3 mg); $[\alpha]^{24}_D$ +33.2 (*c* 0.5, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 215.5 (3.76) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−1.0), 218 (7.1), 20 (2.5); IR (KBr) ν_{max} 3482 (OH), 2926, 2855 (CH), 1757, 1750, and 1717 (C═O), 1245 (CH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 5; ($CDCl_3$, 150 MHz), see Table 6; EIMS *m/z* 604 [M − $HOCOCH_3$], 586 [M − $HOCOCH_3$ − H_2O], 568 [M − $HOCOCH_3$ − $2H_2O$], 550 [M − $HOCOCH_3$ − $3H_2O$], 465, 405, 395, 387, 335, 317, 223, 111; HRMALDITOF MS *m/z* [M + Na]$^+$ 687.4826 (calcd for $C_{39}H_{70}O_8Na$, 687.4812).

Plagioneurin D (8): yellow oil (33.9 mg); $[\alpha]^{24}_D$ +13.9 (*c* 1.0, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 220 (3.63) nm; CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−0.8), 213 (3.2), 206 (3.8); IR (KBr) ν_{max} 3439 (OH), 2925, 2854 (CH), 1740, 1738, 1736 (C═O), 1463, 1246 (CH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 5; ($CDCl_3$, 150 MHz), see Table 6; EIMS *m/z* 620 [M − $HOCOCH_3$], 602 [M − $HOCOCH_3$ − H_2O], 584 [M − $HOCOCH_3$ − $2H_2O$], 566 [M − $HOCOCH_3$ − $3H_2O$], 481, 463, 421, 411, 403, 385, 367, 351, 349, 333, 315, 307, 289, 269, 267, 239, 221, 197, 179, 155, 137; HRESIMS *m/z* [M + Na]$^+$ 703.4805 (calcd for $C_{39}H_{68}O_9Na$, 703.4761).

Plagioneurin E (9): yellow oil (13.8 mg); $[\alpha]^{24}_D$ +17.3 (*c* 1.1, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 222.50 (3.8); CD λ_{max} ($\Delta\epsilon$) (MeOH) 235 (−0.4), 212 (2.8), 205 (3.0); IR (KBr) ν_{max} 3438 (OH), 2924 and 2855 (CH), 1742, 1738, and 1735 (C═O), 1460, 1247 (CH); ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 5; ($CDCl_3$, 150 MHz), see Table 6; EIMS *m/z* 620 [M − $HOCOCH_3$], 602 [M − $HOCOCH_3$ − H_2O], 584 [M − $HOCOCH_3$ − $2H_2O$], 566 [M − $HOCOCH_3$ − $3H_2O$], 481, 463, 421, 411, 403, 385, 367, 351, 349, 333, 315, 307, 289, 269 (20), 267, 239, 221, 197, 179, 155, 137; HRESIMS *m/z* [M + Na]$^+$ 703.4813 (calcd for $C_{39}H_{68}O_9Na$, 703.4761).

MTPA Derivatives of 1, 5, 7, and 8. To each acetogenin (5 mg in 2 mL of CH_2Cl_2) were sequentially added pyridine (1 mL), 4-(dimethylamino)pyridine (4 equiv), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. Each mixture was stirred at rt overnight and eluted with 2−4 mL of CH_2Cl_2; the eluate was washed first using 1% $NaHCO_3$ and second with 0.5% acetic acid. The CH_2Cl_2 layer was dried in vacuo to give the (*S*)-Mosher esters. Use of (*S*)-(+)-α-methoxy-α-(trifloromethyl)phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%. The pertinent ^{1}H NMR chemical shifts of the Mosher esters were recorded (Tables 3, 4, and 7).

Bioassays. The human KB tumor cell line was originally obtained from the ATCC. The cytotoxicity assays were performed according to a published procedure.[14]

Acknowledgment. We express our thanks to A. Gramain, who collected and identified the plant material. This work was supported by a grant from the Centre National de la Recherche Scientifique (CNRS).

References and Notes

(1) Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Eds.; Springer-Verlag: Vienna, 1997; pp 81−307.
(2) Bermejo, A.; Figadère, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269−302.
(3) Zheng, X.; Yang, R.; Xu, R.; Qin, G. *Acta Bot. Sin.* **1997**, *36*, 557−560.
(4) Lavault, M.; Guinaudeau, H.; Bruneton, J.; Sevenet, T.; Hadi, H. A. *Phytochemistry* **1990**, *29*, 3845−3847.
(5) Ee, G. C. L.; Chuah, C. H.; Sha, C. K.; Goh, S. H. *Nat. Prod. Lett.* **1996**, *9*, 141−151.
(6) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *24*, 95−97.
(7) Reiser, M. J.; Hui, Y. H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, V. W.; McLaughlin, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203−10213.
(8) Born, L.; Lieb, F.; Lorentzen, J. P.; Moeschler, H.; M., N.; Söllner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312−316.
(9) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, S.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175−1184.
(10) Defretin, J.; Gleye, C.; Cortes, D.; Franck, X.; Hocquemiller, R.; Figadère, B. *Lett. Org. Chem.* **2004**, *1*, 316−322.
(11) Gu, Z-M.; Fang, X-P.; Zeng, L.; Song, R.; Ng, J. H.; Wood, K. V.; Smith, D. L.; McLaughlin, J. L. *J. Org. Chem.* **1994**, *59*, 3472−3479.
(12) Garwronski, J. K.; Oeveren, A. V.; Van Der. Deen, H.; Leung, C. W.; Feringa, B. L. *J. Org. Chem.* **1996**, *61*, 1513−1515.
(13) Mosher's ester derivatives could not be prepared from **2**, **3**, **4**, **6**, and **9**, because other chemical modifications such as acetylation have been done on these compounds, and after biological evaluation (data not shown), there was not enough quantity to prepare these esters.
(14) Tempête, C.; Werner, G. H.; Favre, F.; Roja, A.; Langlois, N. *Eur. J. Chem.* **1995**, *30*, 647−650.

NP060168J