Six Triterpenoid Saponins from *Maesa laxiflora*

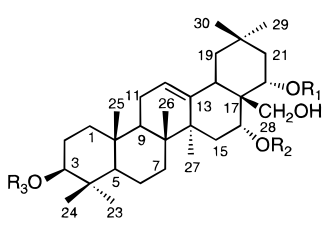
Zhong Jiang,[†] Jean-François Gallard,[†] Marie-Thérèse Adeline,[†] Vincent Dumontet,[†] Mai Van Tri,[‡] Thierry Sévenet,[†] and Mary Païs*[,†]

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, Cedex, France, and Institut de Chimie, NCST, Nghia Do, Cau Giay, Hanoi, Vietnam

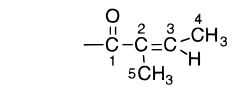
Received January 22, 1999

Six new triterpenoid saponins, maelaxins A−F (**1**−**6**), were isolated from a *n*-BuOH extract of the leaves of *Maesa laxiflora*. They possess 22-*O*-angeloyl-camelliagenin A, 16-*O*-acetyl, 22-*O*-angeloyl-camelliagenin A, or 22-*O*-(2*Z*)-hexenoyl-camelliagenin A as the aglycon. The pentasaccharide moiety linked to C-3 of the aglycon consists of D-glucuronic acid, L-rhamnose, D-glucose, and/or D-galactose. Their structures were elucidated by extensive NMR experiments including ^{1}H−^{1}H (COSY, 2D HOHAHA, NOESY) and ^{1}H−^{13}C (HMQC and HMBC) spectroscopy and chemical evidence.

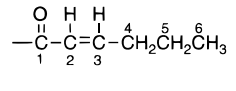
The saponins so far isolated from the genus *Maesa* show virucidal activity, fungistatic and antimutagenic properties,[1] and piscicidal activity.[2] *Maesa laxiflora* Pitard (Myrsinaceae), collected in the framework of a collaboration program between CNRS (France) and NCST (Vietnam), is a small tree that grows in Vietnam and southern regions of the People's Republic of China, where it is used as a traditional medicine. Preliminary biological tests indicated that the EtOH extract of the leaves of *Maesa laxiflora* exhibited moderate cytotoxic activity (IC_{50} 10 μg/mL) against A-549 cells and inhibition of the assembly of tubulin into microtubules (41%, 10 mg/mL). Our investigation on the *n*-BuOH extract of the leaves of this plant led to the isolation of six new triterpenoid saponins, named maelaxins A−F (**1**−**6**), respectively. This paper describes the isolation and structure elucidation of these six new saponins. All of the isolated saponins possess a pentasaccharide moiety linked to C-3 of the aglycon.



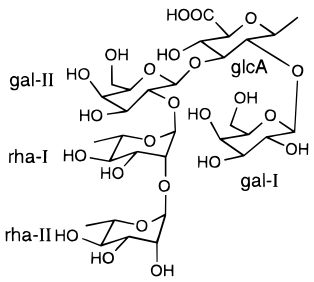
	R_1	R_2	R_3
1	A	H	C
2	A	H	D
3	A	Ac	C
4	A	Ac	D
5	B	H	C
6	B	H	D



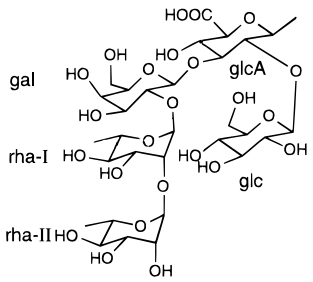
A



B



C



D

Results and Discussion

The EtOH extract of dried leaves of *M. laxiflora* was successively defatted with heptane and EtOAc, then partitioned between *n*-BuOH and water. The *n*-BuOH-soluble fraction was repeatedly subjected to Si gel chromatography to furnish the crude saponins. Reversed-phase HPLC gave six pure saponins, maelaxins A−F (**1**−**6**).

Maelaxin A (**1**) was obtained as a white amorphous powder. The molecular formula was established as $C_{65}H_{104}O_{29}$ on the basis of ^{13}C NMR, ^{13}C DEPT NMR, and MS. Positive-ion FABMS and ESMS gave a $[M + 2Na - H]^+$ ion at *m/z* 1393 and a $[M + Na]^+$ ion at *m/z* 1371. The negative-ion FABMS showed a $[M - H]^-$ anion at *m/z* 1347. Prominent fragment peaks occurred at *m/z* 1201 $[M - H - 146]^-$ and 1185 $[M - H - 162]^-$ due to the independent losses of a deoxyhexose and a hexose unit, indicating a branched oligosaccharide with terminal deoxyhexose and hexose. Other peaks were observed at *m/z* 1055 $[M - H - 146 - 146]^-$, 893 $[M - H - 146 - 146 - 162]^-$, 731 $[M - H - 146 - 146 - 162 - 162]^-$, and 555 $[M - H - 146 - 146 - 162 - 162 - 176]^-$, corresponding to the subsequent loss of a deoxyhexose and a hexose unit and finally a hexuronic acid unit. The peaks at *m/z* 1247 $[M - H - 100]^-$ and 793 $[M - H - 146 - 146 - 162 - 100]^-$ were attributed to the loss of an angeloyl or tigloyl group.[3,4]

The ^{13}C NMR spectrum of **1** showed 65 signals, of which 35 were assigned to a substituted triterpenoid moiety and 30 to the saccharide portion. The chemical shifts of the two olefinic carbons (δ 124.7 and 144.1) and comparison of the remaining chemical shifts (Table 1) with those well established for pentacyclic triterpenes revealed the characteristic carbons of an olean-12-ene triterpene.[5] The methylene resonance at δ 65.0 was diagnostic for the hydroxylated C-28, which correlated with two protons at δ 3.08 and 3.30 in the HMQC spectrum. The absence of a methylene resonance at high field (ca. δ 15.0)[5,6] in the ^{13}C NMR spectrum and the appearance of the signal at δ 4.14 in the ^{1}H NMR spectrum, which correlated with C-28 in the HMBC spectrum, suggested a C-16-OH in **1**. The correlations between H-16 and H-28a,b in the NOESY spectrum pointed to the α-configuration of C-16-OH. The lack of another methylene resonance at ca. δ 33.0 (C-22)[6] and the

* To whom correspondence shoud be addressed. Tel.: +33 1 69 82 30 90. Fax: +33 1 69 07 72 47. E-mail: Mary.Pais@icsn.cnrs-gif.fr.
[†] Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, Cedex, France.
[‡] Institut de Chimie, NCST, Nghia Do, Cau Giay, Hanoi, Vietnam.

10.1021/np990028s CCC: $18.00
Published on Web 05/13/1999

Table 1. NMR Data of the Aglycon Moieties of Compounds **1**−**6** (400 MHz in CD_3OD, J in Hz)[a]

	1, **2**[b]		**3**, **4**[b]		**5**, **6**[b]	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	40.2	1.05	40.0	1.05	40.0	1.05
		1.64		1.64		1.64
2	27.1	2.04	26.9	2.04	26.9	2.04
		1.70		1.70		1.70
3	92.2	3.23	92.0	3.23	92.0	3.22
4	40.7		40.5		40.6	
5	57.2	0.80 dd (11.0, 1.0)	57.0	0.80 dd (11.0, 1.0)	57.0	0.82 dd (11.0, 1.0)
6	19.5	1.60	19.2	1.60	19.3	1.60
		1.42		1.42		1.42
7	34.2	1.35	33.8	1.35	34.0	1.36
8	41.2		41.1		41.0	
9	48.2	1.70	48.2	1.70	48.1	1.68
10	37.9		37.8		37.7	
11	24.8	1.90	24.6	2.02	24.6	1.90
12	124.7	5.37 m	125.9	5.42 m	124.6	5.36 m
13	144.1		143.1		143.9	
14	42.4		42.2		42.2	
15	35.4	1.75	31.7	1.86	35.2	1.73
		1.33		1.50		1.35
16	71.2	4.14 m	74.1	5.24 m	71.8	4.14 m
17	45.5		44.7		45.3	
18	41.9	2.50	41.5	2.60	41.7	2.49
19	48.2	1.10	48.2	1.24	48.1	1.08
		2.53		2.36		2.52
20	32.6		32.4		32.5	
21	42.6	1.55	42.4	1.68	42.4	1.55
		2.29		1.80		2.29
22	74.1	5.47 dd (12.0, 5.6)	72.2	5.45 dd (12.2, 5.8)	73.4	5.45 dd (12.0, 5.6)
23	28.6	1.12 s	28.4	1.10 s	28.3	1.10 s
24	17.1	0.93 s	17.0	0.95 s	17.0	0.91 s
25	16.4	1.00 s	16.2	1.04 s	16.2	1.00 s
26	17.5	0.97 s	17.2	1.03 s	17.3	0.97 s
27	27.9	1.50 s	27.5	1.40 s	27.7	1.48 s
28	65.0	3.08 d (11.0)	64.9	3.22 d (10.8)	64.7	3.04 d (11.0)
		3.30 d (11.0)		3.36 d (10.8)		3.30 d (11.0)
29	33.7	0.93 s	33.7	1.02 s	33.6	0.93 s
30	25.4	1.07 s	25.0	1.08 s	25.2	1.06 s
A (or B)						
1	169.9		169.9		168.3	
2	130.1		129.6		121.4	5.84 d (11.7)
3	138.2	6.09 q (5.8)	138.6	6.04 q (5.8)	151.0	6.28 dt (11.7, 7.2)
4	16.1	2.00 d (5.8)	15.9	2.00 d (5.8)	32.0	2.65 dt (7.2, 6.8)
5	21.1	1.93 s	20.9	1.88 s	23.3	1.46 m
6					14.1	0.97 t (7.2)
*C*OMe			171.4			
CO*Me*			22.1	2.06 s		

[a] Assignments based on 2D NMR experiments. [b] Data in the same column may vary slightly.

appearance of one proton at δ 5.47 (dd, J = 12.0, 5.6 Hz), in combination with the resonances at δ 169.9, 138.2, 130.1, 21.1, and 16.1 in the ^{13}C NMR spectrum, and δ 6.09 (1H, q, J = 5.8 Hz), 2.00 (3H, d, J = 5.8 Hz), and 1.93 (3H, s) in the ^{1}H NMR spectrum, ascertained the presence of a angeloyl moiety rather than a tigloyl moiety in **1**. Tigloyl methyl groups normally appear at higher field in the ^{13}C NMR spectrum (ca. δ 13.0 and 15.0, respectively).[4] The HMBC correlations between the proton at δ 5.47 and C-28, C-16 confirmed that the *O*-angeloyl group should be allocated to C-22. NOESY correlations between H-22 and Me-30,H-28a,b were observed, indicating the α-configuration of the *O*-angeloyl residue. The peak at δ 92.0 in the ^{13}C NMR spectrum, showing a significant glycosidation shift, suggested linkage of the sugar moiety to the aglycon at C-3; H-3 (δ 3.23) correlated with Me-23 in the NOESY spectrum, indicating the usual β-configuration of the C-3-OH.

The ^{13}C NMR spectrum of the sugar portion of **1** exhibited signals for five anomeric carbons (δ 101.0, 101.0, 103.4, 103.9, and 105.9), one carbonyl carbon (δ 175.1), two methylene resonances (δ 62.9 and 63.1), and two methyl groups (δ 18.1 and 18.2). Five anomeric protons (δ 4.52,4.82, 5.00, 5.26, and 5.41) and two methyl doublets (δ 1.29 and 1.31, J = 5.8) were observed in the ^{1}H NMR spectrum of **1**. These observations and comparison of the remaining chemical shifts with those reported in the literature[7] revealed the presence of two rhamnoses, two galactoses, and one glucuronic acid in **1**. This was confirmed by comparison with authentic sugars on co-TLC after acid hydrolysis. The ^{1}H and ^{13}C NMR chemical shift assignments of the sugars were made through ^{1}H−^{1}H COSY, HMQC, HMBC, HOHAHA, and NOESY experiments (Table 2). The positions of the glycosidic linkages were established from the connectivities indicated in the HMBC and NOESY spectra. In the HMBC spectrum, a cross peak between C-3 of the aglycon (δ 92.2) and H-1 of glucuronic acid (δ 4.52) indicated that glucuronic acid (glcA) was connected to C-3 of the aglycon. The linkage of the terminal galactose (gal-I) at C-2 of glcA was indicated by the cross peaks C-2glcA (δ 79.6)/H-1gal-I (δ 4.82), and C-1gal-I (δ 103.4)/H-2glcA (δ 3.93). Similarly, the linkages of the terminal rhamnose (rha-II) at C-2 of the other rhamnose (rha-I), of rha-I at C-2 of the other galactose (gal-II), and of gal-II at the C-3 of glcA were indicated by the cross peaks H-1rha-II (δ 5.00)/C-2rhaI (δ 79.6), C-1rha-II (δ 103.9)/H-2rha-I (δ 3.97),

Table 2. NMR Data of the Sugar Portions of Compounds **1**−**6** (400 MHz in CD_3OD, *J* in Hz)

		1, **3**, **5**[b]		**2**, **4**, **6**[b]	
sugar	position	δ_C	δ_H	δ_C	δ_H
glcA	1	105.9	4.52 d (7.5)	105.9	4.50 d (7.5)
	2	79.6	3.93	80.0	3.95
	3	81.6	4.11	81.3	4.10
	4	72.2	3.62	72.2	3.62
	5	77.1	3.68	72.0	3.72
	6	175.1		175.0	
gal-I (GLC)	1	103.4	4.82 d (7.4)	102.8	4.85 d (7.4)
	2	73.5	3.59	76.2	3.27
	3	75.0	3.50	78.4	3.38
	4	70.3	3.85	72.7	3.12
	5	76.8	3.70	78.1	3.40
	6	63.1	3.62	63.7	3.55
			3.90		3.88
gal-II (gal)	1	101.0	5.26 d (6.7)	101.0	5.22 d (7.0)
	2	76.8	3.77	76.4	3.75
	3	76.0	3.83	76.0	3.83
	4	72.2	3.68	72.2	3.68
	5	76.6	3.59	77.0	3.57
	6	62.9	3.70	62.9	3.65
			3.88		3.84
rha-I	1	101.0	5.41 br s	101.0	5.44 br s
	2	79.6	3.97	79.4	4.00
	3	72.4	3.83	72.3	3.82
	4	74.1	3.40	74.2	3.42
	5	70.3	4.14	70.4	4.12
	6	18.1	1.29 d (5.8)	18.1	1.30 d (5.8)
rha-II	1	103.9	5.00 br s	104.0	5.00 br s
	2	72.2	4.01	72.2	4.02
	3	72.4	3.72	72.4	3.72
	4	74.1	3.40	74.2	3.40
	5	70.3	3.72	70.4	3.72
	6	18.2	1.31 d (5.8)	18.2	1.31 d (5.8)

[a] Assignments based on 2D NMR experiments. [b] Data in the same column may vary slightly.

H-1rha-I (δ 5.41)/C-2gal-II (δ 76.8), C-1rha-I (δ 101.0)/H-2gal-II (δ 3.77), H-1gal-II (δ 5.26)/C-3glcA (δ 81.6), and C-1gal-II (δ 101.0)/H-3glcA (δ 4.11). The NOESY experiment showed cross peaks that were used to define the interglycosidic linkages. Correlations between H-1 and H-5 of glcA, gal-I, and gal-II were observed, indicating β-configurations for these sugars. The J_{H-H} values of the anomeric protons (7.5, 7.4, and 6.7 Hz, respectively) provided further evidence. On the basis of the foregoing, the structure of **1** was deduced as 3-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-22-*O*-angeloyl-camelliagenin A.

Maelaxin B (**2**) showed, in the positive-ion FABMS and ESMS, a $[M + 2Na - H]^+$ ion at *m/z* 1393 and a $[M + Na]^+$ ion at *m/z* 1371. The negative-ion FABMS showed a fragmentation pattern similar to that of **1**. Comparison of the ^1H and ^{13}C NMR data of both compounds indicated identical aglycon moieties. The main differences were the chemical shifts in the sugar portions. In the ^1H NMR spectrum of **2**, there were more signals in the region δ 3.05−3.45; in the ^{13}C NMR spectrum, some of the carbon resonances (δ 78.3 and 78.1) were a little more downfield than those of **1** (δ 77.1 and 76.8). These observations indicated the replacement of one of the galactose units in **2** by a glucose, like a saponin previously isolated from *Maesa ramentacea*.[2] This was confirmed by co-TLC with authentic samples after acid hydrolysis. Correlations in the HMBC and NOESY spectra indicated that the glucose was linked at C-2 of the glcA. The linkages of other sugars were the same as in **1**. Thus, maelaxin B was defined as 3-*O*-{[(α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl (1→3)]-[β-D-glucopyranosyl (1→2)]-β-D-glucuronopyranosyl}-22-*O*-angeloyl-camelliagenin A.

Maelaxin C (**3**) and maelaxin D (**4**) showed, in the positive-ion ESMS, a $[M + 2Na - H]^+$ ion at *m/z* 1435 and a $[M + Na]^+$ ion at *m/z* 1413, 42 mass units higher than those of **1** and **2**, and gave ^{13}C and ^{13}C DEPT NMR data consistent with a $C_{67}H_{106}O_{30}$ molecular formula. Comparison of the ^1H and ^{13}C NMR data of the sugar portion of **3** and **4** with those of **1** and **2** indicated that **3** and **1**, **4** and **2** had the same saccharide chains, respectively. Compounds **3** and **4** varied from **1** and **2** in the aglycon moieties. In the ^1H NMR spectra of **3** and **4**, an additional acetyl methyl singlet appeared at δ 2.06; a signal at δ 5.24 replaced the H-16 signal at δ 4.14. The ^{13}C NMR spectrum of **3** and **4** showed upfield shifts of C-15 (−3.7 ppm) and C-17 (−1.1 ppm) and an additional resonance at δ 22.1, implying the acetylation of C-16-OH.[6] Once again, the HMBC and NOESY experiments of **3** and **4** showed the same glycosidic linkages as in **1** and **2**, respectively. The NOESY correlations H-16 (δ 5.24)/H-28a,b and H-22 (δ 5.45)/H-28a,b indicated the α-configurations of C-16-OAc and C-22-*O*-angeloyl. Thus, the structures of **3** and **4** were identified as 3-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-16-*O*-acetyl, 22-*O*-angeloyl-camelliagenin A and 3-*O*-{[(α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-glucopyranosyl (1→2)]-β-D-glucuronopyranosyl}-16-*O*-acetyl, 22-*O*-angeloyl-camelliagenin A, respectively.

The positive-ion ESMS of maelaxin E (**5**) and maelaxin F (**6**) showed a $[M + 2Na - H]^+$ ion at *m/z* 1407 and a $[M + Na]^+$ ion at *m/z* 1385, 14 mass units higher than those of **1** and **2**, corresponding to the molecular formula $C_{66}H_{106}O_{29}$. The same saccharide chains as those of **1** and **2** were deduced for **5** and **6** by comparison of the NMR data. In the NMR spectra of **5** and **6**, the signals of the angeloyl moiety were replaced by resonances at δ_C 168.3 (carbonyl), 151.0 (methine), 121.4 (methine), 32.0 (methylene), 23.3 (methylene), and 14.1 (methyl) and δ_H 6.28, 5.84, 2.65, 1.46, and 0.97. These observations indicated the existence of a 2-hexenoyl group instead of the angeloyl group in **5** and **6**. This was confirmed by the $^1H-^1H$ COSY, HMQC, and HMBC experiments. The geometric configuration of the double bond was determined as *Z* from the J_{H-H} value (11.7 Hz).[8] A 2-(*Z*)-hexenoyl residue has also been found in the recently reported saponins from *Maesa japonica*.[9] The NOESY correlations H-16 (δ 4.14)/H-28a,b and H-22 (δ 5.45)/H-28a,b indicated the α-configurations of C-16-OH and C-22-*O*-(2*Z*)-hexenoyl. Thus, the structures of **5** and **6** were established as 3-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-22-*O*-(2*Z*)-hexenoyl-camelliagenin A and 3-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-glucopyranosyl (1→2)]-β-D-glucuronopyranosyl}-22-*O*-(2*Z*)-hexenoyl-camelliagenin A, respectively.

Finally, the absolute configurations of the sugars were determined as the D-form for glucuronic acid, glucose, and galactose, and the L-form for rhamnose by the method developed by Hara et al. (see Experimental Section).[10] Maelaxins A−F (**1**−**6**) showed neither cytotoxic properties nor antitubulin activity.

Experimental Section

General Experimental Procedures. Optical rotations at 20 °C were taken on a Perkin−Elmer 241 polarimeter. Spectra

were recorded as follows: IR, Perkin−Elmer Spectrum BX FT-IR system; NMR, Bruker AC 250 (^{13}C NMR and DEPT spectra) and AMX 400 (^{1}H and 2D NMR spectra). ESMS and FABMS were acquired on a VG-ZAB−SEQ spectrometer. Column chromatography: Si gel Merck H60. TLC: Si gel Merck 60 F_{254}. Preparative HPLC: column, Waters Pre-Pak C_{18}, 15 μm, 100 Å, 47 i.d. × 300 mm; eluent, MeOH−H_2O−AcOH (70:30:0.5); flow rate, 50 mL/min; UV detection (220 nm). Semipreparative HPLC: column, Waters Prep Nova-Pak C_{18}, 6 μm, 60 Å, 25 i.d.× 100 mm; eluent, MeOH−H_2O−AcOH (60:40:0.1); flow rate, 6 mL/min; column, Beckman Ultrasphere ODS, 5 μm, 10 i.d.× 250 mm; eluent, CH_3CN−H_2O−AcOH (38:62:0.5); flow rate, 3 mL/min; UV detection (220 nm). Analytical HPLC: column, Nova-Pak ODS C_{18}, 3.9 i.d. × 150 mm; eluent, MeOH−H_2O−AcOH (55:45:0.1), CH_3CN−H_2O−AcOH (33:67:0.5); flow rate, 1 mL/min, UV and DR detections. GC: column, DB-5, J&W Scientific, 0.25 mm i.d. × 30 m; column temperature, 50−230 °C, 15 °C/min then 230 °C, 18 min; carrier gas, N_2.

Plant Material. Leaves of *Maesa laxiflora* Pitard were collected from Yen Chau ("son la"), Vietnam, in February 1996. Identification was confirmed by one of us (V. D.) and Tran Ngoc Ninh, Institut d'Ecologie, NCST, Hanoi, Vietnam. Voucher specimens (VN 065) are deposited at the herbarium of that institute.

Extraction and Isolation. Dried leaves of *M. laxiflora* (1 kg) were extracted exhaustively with EtOH at room temperature. The EtOH extract was defatted with heptane and EtOAc and then partitioned with *n*-BuOH and H_2O to give the *n*-BuOH extract (20.0 g). Repeated Si gel chromatographies eluted with CH_2Cl_2−MeOH−H_2O from 30:10:1 to 12:6:1 gave a crude saponin fraction (4.0 g). Part of the crude saponin (1.8 g) was separated by preparative HPLC into fraction A (820 mg) and fraction B (165 mg). Semipreparative HPLC of 200 mg of fraction A (MeOH−H_2O−AcOH, 60:40:0.1) gave **1** (73 mg, t_R 73.2 min) and **2** (36 mg, t_R 83.3 min). Semipreparative HPLC of fraction B (CH_3CN−H_2O−AcOH, 38:62:0.5) gave **3** (29 mg, t_R 49.0 min), **4** (23 mg, t_R 52.0 min), **5** (15 mg, t_R 60.2 min), and **6** (24 mg, t_R 65.4 min).

Maelaxin A (1): white amorphous powder, mp 245−248 °C, $[\alpha]^{20}_D$ −24.6° (*c* 1.4, MeOH); IR (KBr) ν_{max} 3426, 2925, 1681, 1636, 1384, 1081 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1393 $[M + 2Na - H]^+$ (100), 1371 $[M + Na]^+$ (82); positive-ion FABMS *m/z* 1393 $[M + 2Na - H]^+$ (100); negative-ion FABMS *m/z* 1347 $[M - H]^-$ (100), 1247 (6), 1201 (13), 1185 (9), 1055 (13), 893 (20), 793 (12), 731 (6), 555 (8).

Maelaxin B (2): white amorphous powder, mp 242−245 °C, $[\alpha]^{25}_D$ −16.3° (*c* 1.5, MeOH); IR (KBr) ν_{max} 3422, 2926, 1683, 1632, 1384, 1076 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1393 $[M + 2Na - H]^+$ (44), 1371 $[M + Na]^+$ (100); positive-ion FABMS *m/z* 1393 $[M + 2Na - H]^+$ (100), 1371 $[M + Na]^+$ (37), 1247 (13); negative-ion FABMS *m/z* 1347 $[M - H]^-$ (100), 1201 (13), 1185 (7), 1055 (20), 893 (45), 793 (5), 731 (10), 555 (6).

Maelaxin C (3): white amorphous powder, mp 246−248 °C, $[\alpha]^{25}_D$ −18.2° (*c* 1.4, MeOH); IR (KBr) ν_{max} 3427, 2924, 1716, 1634, 1384, 1073 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1435 $[M + 2Na - H]^+$ (31), 1413 $[M + Na]^+$ (100).

Maelaxin D (4): white amorphous powder, mp 247−249 °C, $[\alpha]^{25}_D$ −15.6° (*c* 1.6, MeOH); IR (KBr) ν_{max} 3425, 2926, 1710, 1635, 1384, 1074 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1435 $[M + 2Na - H]^+$ (50), 1413 $[M + Na]^+$ (100).

Maelaxin E (5): white amorphous powder, mp 256−258 °C, $[\alpha]^{25}_D$ −5.6° (*c* 1.8, MeOH); IR (KBr) ν_{max} 3420, 2926, 1698, 1622, 1384, 1074 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1407 $[M + 2Na - H]^+$ (100), 1385 $[M + Na]^+$ (20).

Maelaxin F (6): white amorphous powder, mp 258−260 °C, $[\alpha]^{25}_D$ −10.0° (*c* 1.4, MeOH); IR (KBr) ν_{max} 3419, 2927, 1700, 1620, 1385, 1075 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ESMS *m/z* 1407 $[M + 2Na - H]^+$ (77), 1385 $[M + Na]^+$ (100).

Acid Hydrolysis of 1. Compound **1** (2 mg) was refluxed with 7% HCl (1 mL) for 3 h. The reaction mixture was partitioned between EtOAc and H_2O. The H_2O layer was concentrated and checked by TLC (Si gel), together with the authentic sugar samples (CH_2Cl_2−MeOH−H_2O, 6.5:4.0:0.8; R_f, 0.20 for glcA, 0.60 for gal, 0.76 for rha).

Acid Hydrolysis of 2. Compound **2** (2 mg) was refluxed with 7% HCl (1 mL) for 3 h. The reaction mixture was partitioned between EtOAc and H_2O. The H_2O layer was concentrated and checked by TLC (Si gel) together with the authentic sugar samples (CH_2Cl_2−MeOH−H_2O, 6.5:4.0:0.8); R_f, 0.2 for glcA, 0.64 for glc, 0.60 for gal, 0.76 for rha).

Determination of the Absolute Configuration of Sugars. A mixture of **1** and **2** (5 mg) was hydrolyzed to give the sugar mixture, which was methylated with trimethyldiazomethane in MeOH. After being dried, the residue was dissolved in MeOH (2 mL) and treated with $NaBH_4$ (20 mg) at room temperature for 30 min. The mixture was passed through a Waters SEP−PAK C_{18} cartridge column (Milford, MA) eluted with H_2O (5 mL) and then MeOH−H_2O (1:1, 5 mL). The MeOH−H_2O (1:1) eluent was dried in vacuo and then dissolved in dry pyridine (1 mL), and 3 mg l-cysteine was added. The mixture was heated at 60 °C for 2 h, dried in vacuo, and trimethysilylated with *N*,*O*-bis(trimethylsilyl)acetamide (0.1 mL) at 70 °C for 0.5 h. After partition between heptane and H_2O, the heptane extract was analyzed by GC. The sugar derivatives showed t_R of 19.62, 24.04, and 24.93 min, identical to those of authentic L-rha, D-glc, and D-gal, respectively. Under the same conditions, derivatives of L-glc and L-gal showed t_R of 25.01 and 26.18 min, respectively.

Bioassays. The cytotoxicity test against A-549 cell line (NCI) was performed using a standard MTT-based colorimetric assay.[11] Tubulin assembly assay: the tubulin solution was prepared and the polymerization was initiated (by a temperature shift from 6 to 37 °C) as described previously.[12] The polymerization rate was calculated from the slope of the increase of turbidity measured as a function of time.[12] The indicated concentration (10 mg/mL) of the inhibitor added to the cold (6 °C) tubulin solution decreased the rate by 41%. The control used for comparison is colchicine (rate decrease: 50% at 1 μg/mL).

Acknowledgment. Thanks are expressed to V. Guerineau for his assistance in HPLC and to B. Hill, Centre de Recherche Pierre Fabre, Castres, France, for the biological assay results. One of the authors (Z.J.) expresses his gratitute to Dr. P. Potier, ICSN, CNRS, Gif-sur-Yvette, France, for fellowship support.

References and Notes

(1) Sindambiwe, J. B.; Calomme, M.; Geerts, S.; Pieters, L.; Vlietinck, A. J.; Vanden Berghe, D. A. *J. Nat. Prod.* **1998**, *61*, 585−590.
(2) Tuntiwachwuttikul, P.; Pancharoen, O.; Mahabusarakam, W.; Wiriyachitra, P.; Taylor, W. C.; Bubb, W. A.; Towers, G. H. N. *Phytochemistry* **1997**, *44*, 491−495.
(3) Chakravarty, A. K.; Das, B.; Pakrashi, S. C. *Phytochemistry* **1987**, *26*, 2345−2349.
(4) Yoshikawa, K.; Taninaka, H.; Kan, Y.; Arihara, S. *Chem. Pharm. Bull.* **1994**, *42*, 2023−2027.
(5) Tori, K.; Seo, S.; Shimaoka, A.; Tomita, Y. *Tetrahedron Lett.* **1974**, 4227−4231.
(6) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(7) Sindambiwe, J. B.; Baldé, A. M.; De Bruyne, T.; Pieters, L.; Van Den Heuvel, H.; Claeys, M.; Vanden Berghe, D. A.; Vlietinck, A. J. *Phytochemistry* **1996**, *41*, 269−277.
(8) Jackman, L. M.; Sternhell, S. In *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon: Oxford, UK, 1969; Part 4, p 302.
(9) Koike, K.; Kudo, M.; Jia, Z.; Nikaido, T.; Ide, Y.; Sakura, T. *J. Nat. Prod.* **1999**, *62*, 228−232.
(10) Hara S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−507.
(11) Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(12) Zavala, F.; Guénard, D.; Potier, P. *Experientia* **1978**, *34*, 1497−1499.

NP990028S