Novel Cytotoxic 4-Phenylfuranocoumarins from *Calophyllum dispar*

David Guilet,[†] Jean-Jacques Hélesbeux,[†] Denis Séraphin,[†] Thierry Sévenet,[‡] Pascal Richomme,*[,†] and Jean Bruneton[†]

SONAS, UFR des Sciences Pharmaceutiques et Ingénierie de la Santé, 16 Bd Daviers, F-49100 Angers, France, and ICSN-CNRS, Gif-sur-Yvette, F-91191, France

Received November 2, 2000

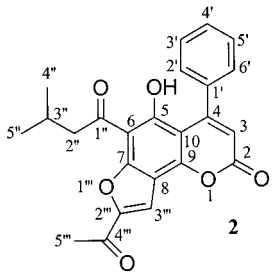
Eight new 4-phenylfuranocoumarins (**1**−**8**) have been isolated from the stem bark and the fruits of *Calophyllum dispar*, together with three known coumarins. The structures of **1**−**8** were established by means of spectroscopic analysis, including extensive 2D NMR studies. Some of these furanocoumarins exhibited significant cytotoxic activity against KB cells.

Numerous species of the genus *Calophyllum* have been shown to contain coumarins in their bark and fruits.[1−6] In addition to their chemotaxonomic interest,[6,7] these coumarins also exhibit significant biological effects such as molluscicidal,[8] piscicidal,[9] and anti-HIV[10,11] activities. As part of our phytochemical study of *Calophyllum* species,[12] we have described previously the isolation of new 4-phenylcoumarins from *Calophyllum dispar* P. F. Stevens.[13] In the present work, eight new 4-phenylfuranocoumarins (**1**−**8**) along with three known coumarins (**9**−**11**) were isolated and characterized from the stem bark and fruits of the same species An evaluation of their cytotoxic properties against KB cells is also presented.

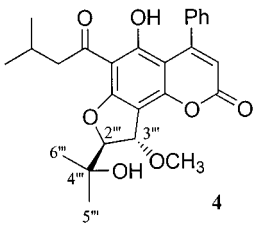
Results and Discussion

Successive purification of an EtOAc-soluble extract of the stem bark of *C. dispar* by repeated chromatography afforded eight coumarins (**1**−**6**, **9**, and **10**), among which compounds **1**−**6** are new.

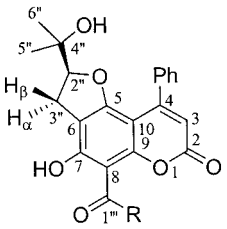
The molecular formula of compound **1** ($C_{22}H_{18}O_5$) was established by HRAPCIMS analysis of its protonated molecule $[M + H]^+$ at *m/z* 363.1248 (Δ +1.6 mmu). The UV spectrum of **1** showed maxima at 238, 283, and 330 nm, similar to those of a 5,7-dioxygenated coumarin.[14,15] Furthermore, a bathochromic shift of an absorption generally associated with 6-acyl-7,8-annulated-5-hydroxycoumarins[16] appeared after addition of NaOH (Table 1). The ^{1}H NMR spectrum of **1** showed a phenolic hydroxyl signal [δ_H 14.79, 1H, s (OH-5)], exchangeable with D_2O, and probably strongly chelated by the carbonyl function of an acyl group. The characteristic singlet of H-3 of a 4-substituted coumarin was observed also at δ_H 6.17 ppm. In the IR spectrum of **1**, absorptions at 756 and 700 cm^{-1} revealed the presence of a monosubstituted phenyl group, whereas five aromatic proton signals [δ_H 7.37, 2H, m (H-2′ and H-6′) and 7.43, 3H, m (H-3′, H-4′, and H-5′)] were in evidence in the ^{1}H NMR spectrum of **1**. The long-range coupling observed in the HMBC spectrum of **1** between H-3 and the quaternary aromatic carbon [δ_C 138.9 (C-1′)] then supported the localization of this phenyl at the 4-position of the coumarin (Figure 1). Examination of the contour map of a gradient-selected DQF-COSY experiment also showed the presence of a *sec*-butyl chain [δ_H 0.97, 3H, t, J = 7.5 Hz (H-5″); 1.24, 3H, d, J = 6.5 Hz (H-3″); 1.55, 1H, m (H-4″); 1.86, 1H, m (H-4″) and 3.83, 1H, m (H-2″)]. Long-range



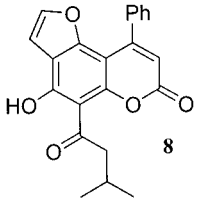
2



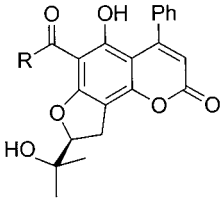
4



5 R = $CH_2CH(CH_3)_2$
6 R = $CHCH_3CH_2CH_3$
7 R = $CH_2CH_2CH_3$



8



9 R = $CH_2CH(CH_3)_2$
10 R = $CHCH_3CH_2CH_3$
11 R = $CH_2CH_2CH_3$

^{1}H−^{13}C couplings then revealed that this fragment was linked to the heterocycle via a carbonyl function [δ_C 208.6 (C-1″)]. The corresponding 2-methyl-1-oxobutyl substituent was thus placed at the 6-position according to the aforementioned UV data. The remaining signals in the ^{1}H NMR spectrum of **1** appeared as a pair of two weakly coupled doublets [δ_H 7.17, 1H, d, J = 2.0 Hz (H-3‴) and 7.66, 1H, d, J = 2.0 Hz (H-2‴)] which could be associated with an unsubstituted furan ring including the last oxygenated function of the coumarin. Assignments of the ^{1}H and ^{13}C NMR resonances of compound **1** (Tables 2 and 3), which we have named disparfuran B, were determined through analysis of its NOESY, HMQC, and HMBC data (Figure 1).

* To whom correspondence should be addressed. Tel: (33) 2-41-22-66-58. Fax: (33) 2-41-48-67-33. E-mail: pascal.richomme@univ-angers.fr.
[†] SONAS.
[‡] ICSN-CNRS.

10.1021/np000517o CCC: $20.00
Published on Web 04/13/2001

Table 1. UV Data for 6-Acyl- (**1**−**4**) and 8-Acylfuranocoumarins (**5**−**8**)

compound[a]		λ_{max} nm (log ϵ)					
1	a	238 (3.78)		283 (4.00)	330 (3.54)		
	b		255 (3.87)	282 (3.76)	314 (3.70)		419 (3.46)
2	a			289 (3.87)		363 (3.34)	434 (2.72)
	b		252 (3.73)	286 (3.66)		374 (3.71)	432 (3.56)
3	a	230 (3.79)		283 (4.04)	346 (3.61)		
	b		250 (3.93)	284 (3.79)	315 (3.74)		428 (3.64)
4	a		245 (4.01)	282 (4.04)	349 (3.65)		
	b		241 (3.89)	282 (4.09)	328 (3.75)		414 (3.12)
5	a	226 (4.10)	234 (4.10)	300 (4.11)	327 (3.87)		
	b		252 (4.10)	283 (3.7)		389 (3.97)	
6	a	227 (4.21)	235 (4.19)	299 (4.22)	325 (4.02)		
	b		252 (4.24)	283 (3.90)		389 (4.07)	
7	a	227 (4.30)	235 (4.28)	299 (4.31)	331 (4.03)		
	b		253 (4.32)	282 (3.92)		389 (4.18)	
8	a	230 (3.96)	253 (3.97)	287 (3.84)	337 (3.58)		
	b	241 (4.01)	259 (3.90)	280 (3.79)	308 (3.58)		

[a] UV spectrum recorded a = in EtOH, and b = in EtOH with NaOH.

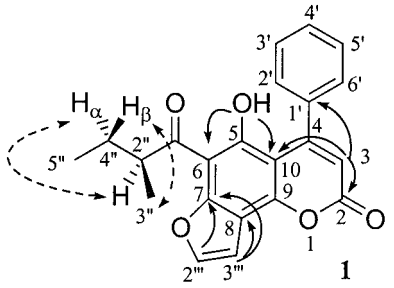


Figure 1. Significant correlations observed in the HMBC (→) and NOESY (↔) NMR spectra of **1**.

The HRFABMS of compound **2** showed a deprotonated molecule $[M - H]^-$ at *m/z* 403.1186 (Δ +0.4 mmu) corresponding to $C_{24}H_{20}O_6$. A chelated phenolic hydroxyl [δ_H 15.05, 1H, s (OH-5)] and a 3-methyl-1-oxobutyl chain [δ_H 1.07, 6H, d, J = 6.5 Hz (H-4″ and H-5″); 2.29, 1H, m (H-3″) and 3.18, 2H, d, J = 6.5 Hz (H-2″) and δ_C 204.4 (C-1″)] could be characterized easily from the NMR data analysis of **2**. However, in contrast to other coumarins belonging to the same series (Table 1), the UV data of **2** did not allow us to assign the position of this acyl chain. Nevertheless, the long-range $^1H-^{13}C$ couplings deduced from the HMBC spectrum of **2** revealed that H-3 [δ_H 6.22] and OH-5 both correlated with the same quaternary carbon C-10 [δ_C 103.4], supporting substitution of the 5- and 6-positions of the coumarin nucleus with a hydroxyl and an acyl chain, respectively. Compound **2** also differed from **1** in the presence of an acetyl group [δ_H 2.63, 3H, s (H-5‴) and δ_C 186.1 (C-4‴) and 26.4 (C-5‴)]. On the basis of the HMBC analysis of **2**, this acetyl moiety appeared as linked to the molecule via a quaternary sp^2 carbon [δ_C 152.3 (C-2‴)]. On further examination of the long-range correlations observed between the proton signal [δ_H 7.83, 1H, s (H-3‴)] and the aromatic quaternary carbons of the coumarin nucleus [δ_C 147.0 (C-7) and 110.0 (C-8)] on one hand, and the sp^2 carbon C-2‴ on the other, an α-acetylfuran structure was evident. Complete assignments of the ^1H and ^{13}C NMR signals of compound **2** (Tables 2 and 3), which we have named disparacetylfuran A, were finally deduced from its HMQC and HMBC data.

Compound **3** was obtained as an amorphous residue, and its molecular formula ($C_{25}H_{24}O_5$) was established by HREIMS (Δ +1.3 mmu). The bathochromic shifts with alkaline of its UV spectrum suggested that **3** contains a 6-acyl-5-hydroxy-7-oxycoumarin chromophore. The NMR data of **3** then revealed the presence of a monosubstituted phenyl [δ_H 7.32, 2H, m (H-2′ and H-6′) and 7.41, 3H, m (H-3′, H-4′, and H-5′)], a chelated phenolic hydroxyl [δ_H 14.55, 1H, s (OH-5)], and a 3-methyl-1-oxobutyl chain [δ_H 0.96, 6H, d, J = 6.5 Hz (H-4″ and H-5″); 2.18, 1H, m (H-3″) and 2.90, 2H, dd, J = 3.0 and 7.0 Hz (H-2″) and δ_C 205.3 (C-1″)] at positions 4, 5, and 6 of the coumarin, respectively. Furthermore, from interlocking DQF-COSY, HMQC, and HMBC data, a 3-methylbut-3-enyl moiety was firmly characterized, with signals at δ_C 89.1 (C-2‴), 30.5 (C-3‴), 142.2 (C-4‴), 113.2 (C-5‴), and 17.1 ppm (C-6‴). In addition, the downfield resonance of C-2‴ was indicative of its substitution by an oxygen atom. Thus, according to the molecular formula of **3**, C-3‴ and C-2‴ should be incorporated in a dihydrofuran structure, including the remaining oxygenated function of the coumarin. Therefore, this compound, whose relative stereochemistry was deduced from a NOESY experiment (Figure 2), was identified as structure **3** and so is closely related to mammea A/AA cyclo F (**9**), previously isolated from *Mammea americana*.[17] We thus propose the trivial name mammea A/AA deshydrocyclo F for compound **3**.

Compound **4** exhibited, in its HRLSIMS, a protonated molecule $[M + H]^+$ at *m/z* 453.1920 associated with the molecular formula $C_{26}H_{28}O_7$ (Δ +0.7 mmu). The UV spectrum of **4** showed bathochromic shifts similar to those of a 6-acyl-5-hydroxy-7-oxycoumarin chromophore (Table 1), and, as for **2** and **3**, a 3-methyl-1-oxobutyl chain was in evidence from the ^1H NMR spectrum of **4** (Tables 2 and 3). As already observed, and due to a strong chelation with the adjacent carbonyl of this acyl chain, the OH-5 proton resonated downfield at δ_H 14.75 ppm. The first difference observed between **3** and **4** concerned the cyclized portion of the structure since an aliphatic methoxyl [δ_H 3.64, 3H, s (OMe-3‴)] appeared in the ^1H NMR spectrum of the latter. The HMBC spectrum of compound **4** then showed that this methoxyl was substituted at the 3‴-position [δ_H 5.23, 1H, d, J = 3 Hz (H-3‴) and δ_C 78.6 (C-3‴)] of the dihydrofuran ring. The second difference was related to the substituent at C-2‴, which was identified in **4** as a 1-hydroxy-1-methylethyl moiety [δ_H 1.35, 3H, s (H-5‴) and 1.39, 3H, s (H-6‴) and δ_C 71.2 (C-4‴)]. Furthermore, the relative stereochemistry of H-2‴ and H-3‴ (J = 3 Hz) was determined to be *trans*.[18] Therefore, **4** was identified as the OMe-3‴ derivative of mammea A/AA cyclo F (**9**) and was accordingly named mammea A/AA methoxycyclo F.

Compound **5** was obtained as a white crystalline solid (mp 113−115 °C) and had the same molecular formula, $C_{25}H_{26}O_6$, based on the HRFABMS (negative ion mode) analysis of its deprotonated molecule $[M - H]^-$ at *m/z* 421.1674 (Δ +2.3 mmu), as mammea A/AA cyclo F (**9**). The

Table 2. ^{1}H NMR Spectral Data for **1**−**4** in $CDCl_3$[a]

position	1	2	3	4
3	6.17 s	6.22 s	5.94 s	5.99 s
OH-5	14.79 s	15.05 s	14.55 s	14.75 s
2′,6′	7.37 m	7.37 m	7.32 m	7.31 m
3′,5′	7.43 m	7.45 m	7.41 m	7.40 m
4′	7.43 m	7.45 m	7.41 m	7.40 m
2″	3.83 m	3.18 d (6.5)	2.90 dd (3.0/7.0)	2.81 dd (7.0/15.0) 3.00 dd (7.0/15.0)
3″	1.24 d (6.5)	2.29 m	2.18 m	2.21 m
4″	H_α 1.86 m H_β 1.55 m	1.07 d (6.5)	0.96 d (6.5)	0.96 d (7.0)
5″	0.97 t (7.5)	1.07 d (6.5)	0.96 d (6.5)	0.96 d (7.0)
2‴	7.66 d (2.0)		5.48 t (9.0)	4.65 d (3.0)
3‴	7.17 d (2.0)	7.83 s	H_α 3.15 dd (8.0/15.0) H_β 3.50 dd (10.0/15.0)	5.23 d (3.0)
OMe-3‴				3.64 s
5‴		2.63 s	H_a 5.15 s H_b 5.03 s	1.35 s
6‴			1.83 s	1.39 s

[a] *J* values (Hz) are shown in parentheses.

Table 3. ^{13}C NMR Spectral Data for **1**−**4** in $CDCl_3$

position	**1**	**2**	**3**	**4**
2	159.4	158.5	159.7	159.8
3	114.3	115.0	111.9	112.5
4	156.8	156.4	156.5	156.5
5	163.5	165.9	164.5	166.5
6	104.9	119.0	103.2	103.3
7	155.8	147.0	164.5	164.4
8	109.8	110.0	104.5	105.9
9	153.4	156.5	155.6	156.8
10	103.3	103.4	102.2	102.8
1′	138.9	138.4	139.0	139.0
2′,6′	127.1	127.2	127.1	127.2
3′,5′	127.7	127.8	127.5	127.7
4′	128.4	128.6	128.1	128.3
1″	208.6	204.4	205.3	205.1
2″	45.7	51.8	51.7	52.0
3″	16.3	25.5	25.3	25.0
4″	26.5	22.6	22.5	22.6
5″	11.8	22.6	22.5	22.6
2‴	143.8	152.3	89.1	97.7
3‴	104.7	111.2	30.5	78.6
OMe-3‴				57.7
4‴		186.1	142.2	71.2
5‴		26.4	113.2	25.5
6‴			17.1	25.9

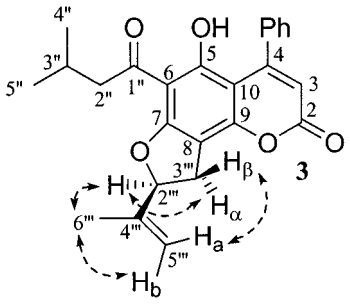


Figure 2. Significant correlations observed in the NOESY NMR spectrum of **3**.

gross features of their ^{1}H and ^{13}C NMR spectra confirmed a close structural relationship between **5** and **9**. Indeed, the same substituents for the coumarin could be characterized in both compounds through DQF-COSY, NOESY, HMQC, and HMBC experiments, namely, a monosubstituted phenyl, a chelated hydroxyl, a 3-methyl-1-oxobutyl group, and an α-(1-hydroxy-1-methylethyl)dihydrofuran moiety [δ_H 0.94, 3H, s (H-6″); 1.02, 3H, s (H-5″); 2.93, 1H, dd, J = 8.5 and 15.5 Hz (H-3″); 3.08, 1H, dd, J = 10.0 and 15.5 Hz (H-3″) and 4.52, 1H, t, J = 9.0 Hz (H-2″)] (Tables 4 and 5). Significant cross-peaks observed in the HMBC and NOESY spectra of **5** are shown in Figure 3. However, **5** and **9** exhibited quite different shifts with alkaline reagents in their UV spectra (Table 1). It then appeared that **5** was a regioisomer of **9**, and additional evidence for the substitution pattern of the coumarin in **5** included the key long-range ^{1}H−^{13}C couplings (Figure 3) observed between the chelated OH-7 (δ_H 14.33) and the heterocyclic carbons at δ_C 110.0 (C-6), 163.7 (C-7), and 105.0 ppm (C-8). The structure of this compound was thus deduced as **5**, which, according to the nomenclature proposed for related compounds,[16,19] we have named mammea A/BA cyclo F.

Compound **6** displayed UV and ^{1}H and ^{13}C NMR data also closely matching those of **5**, with which it shared the same molecular formula $C_{25}H_{26}O_6$ (see Experimental Section). It then appeared from their NMR data that **5** and **6** differed only in the nature of their acyl substituents. As already mentioned, a 2-methyl-1-oxobutyl chain at the 8-position could be characterized from ^{1}H and ^{13}C NMR evidence (Tables 4 and 5). Therefore, **6**, or mammea A/BB cyclo F, was identified as the regioisomer of the mammea A/AB cyclo F (**10**), previously isolated from *M. americana*.[17]

Column chromatography of an EtOAc-soluble extract of the fruits of *C. dispar* yielded two additional new 4-phenylfuranocoumarins, **7** and **8**, together with the known mammea A/AC cyclo F (**11**).[20]

The spectral data (UV, IR, ^{1}H and ^{13}C NMR) recorded for **7** ($C_{24}H_{24}O_6$) indicated certain strong structural similarities with coumarins **5** and **6** (Tables 1, 4, and 5). In this respect, it appeared from NMR evidence that **6** and **7** differed only in their acyl substituents. A 1-oxobutyl acyl chain could be characterized in **7** from the signals at δ_H 1.07 [3H, t, J = 7.5 Hz (H-4‴)], 1.81 [2H, m (H-3‴)] and 3.31 ppm [2H, t, J = 7.0 Hz (H-2‴)] and δ_C 13.8 (C-4‴), 18.0 (C-3‴), 46.5 (C-2‴), and 206.2 ppm (C-1‴). This coumarin, mammea A/BC cyclo F, was thus identified as structure **7**.

The second new compound (**8**) isolated from the fruits of *C. dispar* exhibited in its HREIMS a molecular ion at *m*/*z* 362.1170, which correlated with the molecular formula $C_{22}H_{18}O_5$ (Δ +1.6 mmu). The ^{1}H NMR spectrum of **8** revealed the presence in the molecule of monosubstituted phenyl, 3-methyl-1-oxobutyl, and chelated phenolic hydroxyl functionalities. It also exhibited doublets of two weakly coupled olefinic protons [δ_H 7.31, 1H, d, J = 2.0 Hz (H-2″) and 6.96, 1H, d, J = 2.0 Hz (H-3″)], thus suggesting, as for compound **1**, the presence of a furan moiety in **8**.

Table 4. ^{1}H NMR Spectral Data for **5**−**8** in $CDCl_3$[a]

position	5	6	7	8
3	6.08 s	6.07 s	6.07 s	6.22 s
OH-7	14.33 s	14.32 s	14.29 s	14.84 s
2′, 6′	7.32 m	7.32 m	7.32 m	7.42 m
3′, 5′	7.44 m	7.44 m	7.44 m	7.50 m
4′	7.44 m	7.44 m	7.44 m	7.50 m
2″	4.52 t (9.0)	4.52 t (9.0)	4.52 t (9.0)	7.31 d (2.0)
3″	H_α 3.08 dd (10.0/15.5)	H_α 3.07 dd (10.0/15.0)	H_α 3.08 dd (10.0/15.5)	6.96 d (2.0)
	H_β 2.93 dd (8.5/15.5)	H_β 2.93 dd (8.0/15.0)	H_β 2.93 dd (9.0/15.5)	
5″	1.02 s	1.02 s	1.01 s	
6″	0.94 s	0.94 s	0.94 s	
2‴	3.19 d (7.0)	3.96 m	3.31 t (7.0)	3.30 d (7.0)
3‴	2.32 m	1.28 d (7.0)	1.81 m	2.37 m
4‴	1.08 d (6.5)	1.50 m	1.07 t (7.5)	1.09 d (7.0)
		1.94 m		
5‴	1.08 d (6.5)	1.01 t (7.0)		1.09 d (7.0)

[a] *J* values (Hz) are shown in parentheses.

Table 5. ^{13}C NMR Spectral Data for **5**−**8** in $CDCl_3$

position	5	6	7	8
2	159.1	159.1	159.1	159.2
3	111.0	111.0	111.0	112.0
4	154.9	154.9	154.9	153.7
5	161.9	161.8	161.9	154.2
6	110.0	110.1	110.0	115.0
7	163.7	163.9	163.6	162.7
8	105.0	104.5	104.9	106.1
9	157.3	157.1	157.4	154.3
10	98.6	98.7	98.6	100.0
1′	138.0	138.1	138.0	137.1
2′,6′	127.4	127.4	127.4	127.9
3′,5′	127.9	127.9	127.9	128.1
4′	128.8	128.8	128.8	129.3
2″	92.7	92.6	92.7	146.6
3″	26.8	26.6	26.8	104.9
4″	71.6	71.6	71.6	
5″	23.2	23.2	23.2	
6″	24.8	24.8	24.8	
1‴	206.1	210.4	206.2	207.3
2‴	53.4	46.7	46.5	53.6
3‴	25.6	16.5	18.0	25.6
4‴	22.7	27.1	13.8	22.7
5‴	22.7	11.8		22.7

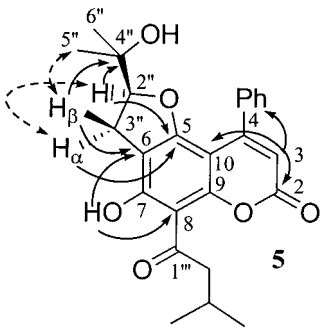


Figure 3. Significant correlations observed in the HMBC (→) and NOESY (↔) NMR spectra of **5**.

The exact localization of the substituents on the coumarin was then deduced from an analysis of the ^{1}H−^{13}C long-range couplings. Complete assignments of the ^{1}H and ^{13}C NMR spectra of **8** were finally completed through DQF-COSY, HMQC, and HMBC experiments (Tables 4 and 5). Therefore, this compound was assigned structure **8** and has been named isodisparfuran A.

Each known compound **9**,[17] **10**,[17] and **11**[20] isolated from *C. dispar*, was identified by NMR (HMQC, HMBC) and MS and by comparison of its spectral data (UV, IR, ^{1}H NMR) with those reported in the literature. The ^{13}C NMR spectra of mammea A/AA cyclo F (**9**) and A/AB cyclo F (**10**) are assigned in the Experimental Section since these data have not been reported previously.

Table 6. Cytotoxicity against KB Cells of Furanocoumarins

	3	5	6	9	10	11
ED_{50} (μg/mL)	40	9	15	6	5	6

It may be noticed that neither new coumarins **1** and **3**−**7** nor known compounds **9**−**11**[17,20] present any significant optical activities, this point having been already stressed for a number of coumarins isolated from the genus *Mammea*.[14−17,21] We thus tried to determine the enantiomeric purity of **9**, available in sufficient amount following biological evaluation tests. Using R-(−)-α-acetoxyphenylacetic acid as the chiral solvating agent,[22] it then appeared in the corresponding ^{1}H NMR spectrum (see Experimental Section) that signals for the furan part of **9** were equally doubled, pointing to a racemic nature of this compound. Due to the small isolated amounts in our hands, this experiment unfortunately could not be repeated, but we may assume that coumpounds **3**−**5**, **7**, **10**, and **11** are racemic also, since their furan moiety is probably a derivative from a spontaneous cyclization of the corresponding prenylated precursors. However, this deduction should not be directly extended to compounds **1** and **6**, where the acyl chain is probably issued from a chiral amino acid such as isoleucin.[23] Furthermore, in this latter series, weak optical activities are reported for highly concentrated solutions.[21,24]

The cytotoxic effect against KB cells of a number of these coumarins was determined (Table 6). Most of the furanocoumarins tested exhibited a significant activity in this assay, especially in the case of the dihydrofuranocoumarins **5**−**7**, which inhibited 50% of the cellular growth at a concentration of 5−6 μg/mL.

It should be noticed that coumarins isolated from *C. dispar* differ from those generally identified in this genus.[1−6] Indeed, most of the *Calophyllum* coumarins show an α,β-unsaturated acyl moiety, which generally undergoes cyclization with an *ortho*-phenol group, thus generating an additional pyran ring in the molecule. The acyl moieties of the coumarins of *C. dispar* are saturated, as observed in other genera such as *Mammea*[14−17,19,21,24] and *Mesua*[20,25,26] (both associated with *Calophyllum* in the subfamily Calophylloideae[27]). Although relationships within *Calophyllum* are not clear,[27] *C. dispar* could nevertheless represent the first member of a minor group showing coumarins with saturated acyl moieties and should then be considered as a link with the other genera in the subfamily Calophylloideae of the Clusiaceae.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal 8100 melting point apparatus and are uncorrected. Optical rotations were measured on a Schmidt-Haensch-polartronic-I polarimeter. IR spectra were recorded on a Perkin-Elmer 580 spectrophotometer, and UV spectra were taken on a Hitachi U-2000 spectrophotometer. HREIMS (70 eV) were recorded on a Varian MAT 311 spectrometer, and HRFABMS and HRAPCIMS were recorded on a JEOL JMS-700 spectrometer. NMR spectra were recorded in $CDCl_3$ solution on a JEOL GSX 270 WB FT spectrometer or a Bruker Avance DRX 500 (2D experiments) instrument, using TMS as the internal standard. Si gel 60 (Macherey-Nagel, 230−400 mesh) was used for column chromatography, and precoated Si gel plates (Macherey-Nagel, SIL G/UV254, 0.25 mm) were used for preparative TLC. The compounds were detected under UV light at 254 and 366 nm.

Plant Material. The stem bark and the fruits of *Calophyllum dispar* P. F. Stevens were collected from Terrangganu, Malaysia, in October, 1994. An herbarium specimen (KL 4430) is deposited at the University of Kuala-Lumpur, Malaysia.

Extraction and Isolation. The dried and powdered stem bark of *C. dispar* (5 kg) was extracted with ethyl acetate (7 L) for 72 h in a Soxhlet apparatus. After concentration, the extract gave a residue (238.85 g). A portion of the residue (87.50 g) was chromatographed over Si gel by medium-pressure liquid chromatography, eluting with *n*-hexane and a 5% stepwise gradient of ethyl acetate (0 to 80%) to afford several fractions, labeled, in order of elution, A to I. Workup of fraction B by repeated column chromatography and preparative TLC using *n*-hexane−ethyl acetate (97:3) led then to the isolation of **1** (2 mg, 0.002%) and **3** (3 mg, 0.003%). Workup of fraction E also by repeated column chromatography and preparative TLC using toluene−ethyl acetate (90:10) afforded **2** (4 mg, 0.005%). Fraction F was chromatographed over Si gel using toluene−ethyl acetate (80:20) and afforded **5** (14 mg, 0.016%) and **6** (55 mg, 0.063%). Fraction G was crystallized from diethyl ether−hexane (70:30) to give **9**[17] (1.43 g, 1.6%), and the supernatant was then chromatographed over Si gel, eluted with toluene−methanol (97:3), and afforded **10**[17] (125 mg, 0.14%) and **4** (1.5 mg, 0.002%).

The same procedure was applied to the dried and powdered fruits of *C. dispar* (140 g). After extraction with ethyl acetate, 4.60 g of residue was obtained and then chromatographed on Si gel by medium-pressure liquid chromatography, eluting with *n*-hexane and a 5% stepwise gradient of ethyl acetate (0 to 80%) to afford several fractions, labeled, in order of elution, A to H. Workup of fraction B by repeated column chromatography and preparative TLC using *n*-hexane−ethyl acetate (97:3) led to the purification of **8** (4 mg, 0.09%). Workup of fraction F, using toluene−ethyl acetate (80:20), yielded **5** (123 mg, 2.67%) and **7** (31 mg, 0.67%). Fraction G was chromatographed over Si gel, eluted with toluene−methanol (97:3), and afforded **9**[17] (88 mg, 1.91%) and **11**[20] (31 mg, 0.67%).

Disparfuran B (5-hydroxy-6-(2-methyl-1-oxobutyl)-4-phenyl-2*H*-furo[2′,3′:5,6]-benzo[1,2-*b*]pyran-2-one, 1): white amorphous solid; $[\alpha]^{25}_D$ 0° (*c* 0.04, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3440, 1732, 1717, 1622, 1559, 756, 700 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRAPCIMS *m/z* 363.1248 ($[M + H]^+$ calcd for $C_{22}H_{19}O_5$, 363.1232); R_f 0.76, *n*-hexane−ethyl acetate (80:20).

Disparacetylfuran A (8-acetyl-5-hydroxy-6-(3-methyl-1-oxobutyl)-4-phenyl-2*H*-furo[2′, 3′:5,6]benzo[1,2-*b*]pyran-2-one, 2): yellow amorphous solid; UV, see Table 1; IR ($CHCl_3$) ν_{max} 3460, 1717, 1684, 1625, 758, 701 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRFABMS *m/z* 403.1186 ($[M - H]^-$ calcd for $C_{24}H_{19}O_6$, 403.1182); R_f 0.40, *n*-hexane−ethyl acetate (80:20).

Mammea A/AA deshydrocyclo F (8,9-dihydro-5-hydroxy-8-(1-methylethylenyl)-6-(3-methyl-1-oxobutyl)-4-phenyl-2*H*-furo[2′,3′:5,6]benzo[1,2-*b*]pyran-2-one, 3): yellow amorphous solid; $[\alpha]^{25}_D$ 0° (*c* 0.06, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3430, 3060, 2960, 1746, 1617, 768, 700 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HREIMS *m/z* 404.1637 ($[M]^+$ calcd for $C_{25}H_{24}O_5$, 404.1624); R_f 0.72, *n*-hexane−ethyl acetate (80:20).

Mammea A/AA methoxycyclo F (8,9-dihydro-5-hydroxy-8-(1-hydroxy-1-methyl-ethyl)-9-methoxy-6-(3-methyl-1-oxobutyl)-4-phenyl-2*H*-furo[2′,3′:5,6]benzo[1,2-*b*]pyran-2-one, 4): yellow amorphous solid; $[\alpha]^{25}_D$ 0° (*c* 0.03, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3410, 1734, 1719, 1617, 768, 699 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRLSIMS *m/z* 453.1920 ($[M + H]^+$ calcd for $C_{26}H_{29}O_7$, 453.1913); R_f 0.44, *n*-hexane−ethyl acetate (70:30).

Mammea A/BA cyclo F (2,3-dihydro-4-hydroxy-2-(1-hydroxy-1-methylethyl)-5-(3-methyl-1-oxobutyl)-9-phenyl-7*H*-furo[2′,3′:3,4]benzo[1,2-*b*]pyran-2-one, 5): white crystals (*n*-hexane−ethyl acetate (90:10); mp 113−115 °C; $[\alpha]^{25}_D$ 0° (*c* 2.0, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3470, 1725, 1605, 1561, 756, 702 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; HRFABMS *m/z* 421.1674 ($[M - H]^-$ calcd for $C_{25}H_{25}O_6$, 421.1651); R_f 0.61, *n*-hexane−ethyl acetate (70:30).

Mammea A/BB cyclo F (2,3-dihydro-4-hydroxy-2-(1-hydroxy-1-methylethyl)-5-(2-methyl-1-oxobutyl)-9-phenyl-7*H*-furo[2′,3′:3,4]benzo[1,2-*b*]pyran-2-one, 6): white amorphous solid; $[\alpha]^{25}_D$ 0° (*c* 0.2, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3460, 1732, 1607, 1559, 758, 704 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; HRFABMS *m/z* 421.1639 ($[M - H]^-$ calcd for $C_{25}H_{25}O_6$, 421.1651); R_f 0.61, *n*-hexane−ethyl acetate (70:30).

Mammea A/BC cyclo F (2,3-dihydro-4-hydroxy-2-(1-hydroxy-1-methylethyl)-5-(1-oxobutyl)-9-phenyl-7*H*-furo-[2′,3′:3,4]benzo[1,2-*b*]pyran-2-one, 7): white amorphous solid; $[\alpha]^{25}_D$ 0° (*c* 0.6, $CHCl_3$); UV, see Table 1; IR ($CHCl_3$) ν_{max} 3460, 1720, 1630, 769, 704 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; HRFABMS *m/z* 407.525 ($[M - H]^-$ calcd for $C_{24}H_{23}O_6$, 407.1495); R_f 0.61, *n*-hexane−ethyl acetate (70:30).

Isodisparfuran A (4-hydroxy-5-(3-methyl-1-oxobutyl)-9-phenyl-7*H*-furo[2′,3′:3,4]-benzo[1,2-*b*]pyran-2-one, 8): white amorphous solid; UV, see Table 1; IR ($CHCl_3$) ν_{max} 3450, 2930, 1744, 1609, 756, 702 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; HREIMS *m/z* 362.1170 ($[M]^+$ calcd for $C_{22}H_{18}O_5$, 362.1154); R_f 0.80, *n*-hexane−ethyl acetate (80:20).

Mammea A/AA cyclo F (9): ^1H NMR (0.03 M in $CDCl_3$ + 0.3 M of *R*-(−)-α-acetoxyphenylacetic acid, 500 MHz), δ_H 4.92 and 4.91 (1H, t, J = 9.0 Hz, H-2‴), 3.32 and 3.31 (2H, d, J = 9.0 Hz, H-3‴), 1.44 and 1.43 (3H, s, H-5‴), 1.34 and 1.33 (3H, s, H-6‴); ^{13}C NMR ($CDCl_3$, 67.5 MHz), δ_C 22.6 (C-4″ and C-5″), 24.9 (C-3‴), 25.0 (C-3″), 26.3 (C-2‴), 26.6 (C-9), 51.9 (C-2″), 71.4 (C-1‴), 92.9 (C-8), 102.3 (C-4a), 103.3 (C-6), 105.1 (C-9a), 111.8 (C-3), 127.2 (C-2′ and C-6′), 127.5 (C-3′ and C-5′), 128.2 (C-4′), 138.9 (C-1′), 155.5 (C-9b), 156.6 (C-4), 159.8 (C-2), 164.2 (C-6a), 164.3 (C-5), 205.2 (C-1″).

Mammea A/AB cyclo F (10): ^{13}C NMR ($CDCl_3$, 67.5 MHz), δ_C 11.7 (C-5″), 16.1 (C-3″), 24.9 (C-3″), 26.0 (C-4″), 26.2 (C-2‴), 26.5 (C-9), 45.5 (C-2″), 71.3 (C-1‴), 92.9 (C-8), 102.1 (C-4a), 102.7 (C-6), 105.2 (C-9a), 111.5 (C-3), 127.1 (C-2′ and C-6′), 127.5 (C-3′ and C-5′), 128.1 (C-4′), 138.8 (C-1′), 155.3 (C-9b), 156.7 (C-4), 159.8 (C-2), 163.9 (C-6a), 164.7 (C-5), 209.5 (C-1″).

Cytotoxicity Testing. The cytotoxicity assays were carried out in triplicate against human nasopharyngeal carcinoma KB cell lines using a modification of the published method.[28] After 72 h incubation at 37 °C with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. The cultured cells were treated at five concentrations of pure test compounds ranging from 50 to 0.01 μg/mL. Optical density was determined at 540 nm on a Titertek Multiscan photometer. The ED_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Doxorubicin, as positive control substance, presented an ED_{50} value of 0.058 μg/mL.

Acknowledgment. The authors express their thanks to C. Gaspard (GDR "Substances d'intérêt biologique", ICSN, Gif-Sur-Yvette, France), who performed the cytotoxicity evaluation on KB cells.

References and Notes

(1) Nigam, S. K.; Mitra, C. R.; Kunesch, G.; Polonsky, J. *Tetrahedron Lett*. **1967**, *28*, 2633−2636.
(2) McKee, C.; Fuller, M.; Covington, C. D.; Cardellina, J. H., II; Gulakowski, R. J.; Krepps, B. L.;. McMahon, J. B.; Boyd, M. R. *J. Nat. Prod*. **1996**, *59*, 754−758.
(3) Somanathan, R.; Sultanbawa, M. U. S. *J. Chem. Soc., Perkin Trans. I* **1972**, 1935−1943.
(4) Cao, S. G.; Wu, X. U.; Sim, K. Y.; Tan, B. H. K.; Vittal, J. L.; Pereira, J. T.; Goh, S. H. *Helv. Chem. Acta* **1998**, *81*, 1404−1416.
(5) McKee, T. C.; Covington, C. D.; Fuller, R. W.; Bokesch, H. R.; Young, S.; Cardellina, J. H.; Kadushin, M. R.; Soejarto, D. D.; Stevens, P. F.; Cragg, G. M.; Boyd, M. R. *J. Nat. Prod*. **1998**, *61*, 1252−1256.
(6) Guilet, D. Étude phytochimique d'une Clusiaceae malaise, le *Calophyllum dispar*. Ph.D. Thesis, Angers University, Angers, France, 291, 2000; pp 23−55 and 215−225.
(7) Ramiandrasoa, F.; Kunesch, N.; Poisson, J. *Tetrahedron* **1983**, *39*, 3923−3928.
(8) Ravelonjato, B.; Libot, F.; Ramiandrasoa, F.; Kunesch, N.; Gayral, P.; Poisson, J. E. *Planta Med*. **1992**, *58*, 51−55.
(9) Kawazu, K.; Ohigashi, H.; Takahashi, N.; Mitsui, T. *Bull. Inst. Chem. Res., Kyoto Univ*. **1972**, *50*, 160−167.
(10) Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H.; MacMahon, J. B.; Currens, M. J.; Buckheit, R. W.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem*. **1992**, *35*, 2735−2743.
(11) Spino, C.; Dodier, M.; Sotheeswaran, S. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3475−3478.
(12) Morel, C.; Séraphin, D.; Oger, J. M.; Litaudon, M.; Sévenet, T.; Richomme, P.; Bruneton, J. *J. Nat. Prod*. **2000**, *63*, 1471−1474.
(13) Guilet, D.; Morel, C.; Séraphin, D.; Sévenet, T.; Wiart, C.; Hadi, A. H. A.; Richomme, P.; Bruneton, J. *Heterocycles* **1999**, *51*, 67−76.
(14) Crombie, L.; Games, D. E.; McCormick, A. *J. Chem. Soc.* (*C*) **1967**, 2553−2558.
(15) Carpenter, I.; McGarry, E. J.; Scheinmann, F. *J. Chem. Soc.* (*C*) **1971**, 3783−3790.
(16) Crombie, L.; Jones, R. C. F.; Palmer, C. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 317−331.
(17) Crombie, L.; Games, D. E.; Haskins, N. J.; Reed, G. F. *Tetrahedron Lett*. **1970**, 3979−3982.
(18) Kofinas, C.; Chinou, I.; Loukis, A.; Harvala, C.; Maillard, M.; Hostettmann, K. *Phytochemistry* **1998**, *48*, 637−641.
(19) Crombie, L.; Games, D. E.; McCormick, A. *Tetrahedron Lett*. **1966**, 151−156.
(20) Morel, C.; Guilet, D.; Oger, J. M.; Séraphin, D.; Sévenet, T.; Wiart, C.; Hadi, A. H. A.; Richomme, P.; Bruneton, J. *Phytochemistry* **1999**, *50*, 1243−1247.
(21) Crichton, E. G.; Waterman, P. G. *Phytochemistry* **1978**, *17*, 1783−1786.
(22) Parker, D. *Chem. Rev*. **1991**, *91*, 1441−1457.
(23) Kunesch, G.; Polonsky, J. *Phytochemistry* **1969**, *8*, 1221−1226.
(24) Finnegan, R. A.; Merkel, K. E.; Back, N. *J. Pharm. Sci*. **1972**, *61*, 1599−1603.
(25) Chakraborty, D. P.; Chatterji, D. *J. Org. Chem*. **1969**, *34*, 3784−3786.
(26) Govindachari, T. R.; Pai, B.; Subramaniam, P.; Rao, U.; Muthukumaraswamy, N. *Tetrahedron* **1967**, *23*, 4161−4165.
(27) Stevens, P. F. *J. Arnold Arbor*. **1980**, *61*, 117−690.
(28) Mosmann, T. *J. Immunol. Method* **1983**, *65*, 55−63.

NP000517O