Pregnane Glycosides from *Leptadenia pyrotechnica*

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The whole plant of *Leptadenia pyrotechnica* afforded 18 new pregnane glycosides (**1**−**18**) with sarcostin, 11-hydroxysarcostin, and deacetylmetaplexigenin as the aglycon moieties and acetyl, benzoyl, cinnamoyl, *p*-coumaroyl, and nicotinoyl ester moieties linked at C-12 and/or C-20 of the aglycon and hexopyranose, 6-deoxy-3-*O*-methylhexopyranose, and 2,6-dideoxy-3-*O*-methylhexopyranose sugars linked at C-3 of their aglycon. The structures of these compounds were elucidated by spectroscopic data interpretation and from chemical evidence. The antiproliferative activity of all compounds was evaluated using three continuous murine and human culture cell lines, J774.A1, HEK-293, and WEHI-164. Compounds having deacethylmetaplexigenin as aglycon and a cinnamoyl ester moiety linked at C-12 were the most active constituents.

Plants belonging to the family Asclepiadaceae are frequently used in traditional medicine and have been reported to be rich in steroidal glycosides.[1,2] *Leptadenia pyrotechnica* (Forsk.) Decne (Asclepiadaceae) is a plant occurring wild in the sahelian region of West Africa countries from Senegal to Nigeria, where it is used in folk medicine.[3] The leaves and bark of the plant are used in folk medicine in Mali to prepare antispasmodic, antiinflammatory, and antibacterial remedies.[4] A previous study on the aerial parts of the plant led to the isolation of alkaloids, while some pregnane glycosides of which showed cytotoxic activity for cancer cell lines.[5]

The present report deals with the isolation and characterization of 18 new polyhydroxypregnane glycoside esters from the whole plant of *L. pyrotecnica*. The structures of these compounds are based on the aglycons sarcostin, 11-hydroxysarcostin, and deacetylmetaplexigenin and have acetyl, benzoyl, cinnamoyl, *p*-coumaroyl, and nicotinoyl ester moieties linked at C-12 and/or C-20 of their aglycon. In addition, all compounds possess an oligosaccharide chain at C-3 of the aglycon consisting of four to six sugar units. The structures were determined mainly through the use of 1D and 2D NMR and ESIMS^n techniques, as well as by chemical evidence. Taking into account the fact that the pregnane glycosides possess cytotoxic activity against various tumor cell lines,[6] all isolated compounds were tested for their antiproliferative activity against the J774.A1, HEK-293, and WEHI-164 cell lines. Compounds having deacethylmetaplexigenin as aglycon and a cinnamoyl ester moiety linked at C-12 were the most active constituents, confirming the significant cytotoxic activity of pregnane glycosides for cancer cells.

Results and Discussion

Compound **1** (chain A) (Figure 1) was assigned the molecular formula $C_{70}H_{101}NO_{26}$, as determined from the ^{13}C NMR and DEPT data and the ESIMS in the positive-ion mode. In the full mass spectrum a single sodiated molecular ion peak could be detected at *m*/*z* 1394 and a molecular weight of 1371 could be deduced for this compound.[12] To obtain structural information on the molecule, a multistage MS^n analysis was performed. Fragmentation of the *m*/*z* 1394 parent ion generated an MS^2 spectrum showing a largely predominant fragment at *m*/*z* 1246, obtained by a 148 Da neutral loss; subsequent fragmentation of this ion produced a single product at *m*/*z* 1123, and further fragmentation of this species induced two consecutive 162 and 160 Da eliminations, generating fragment ions at *m*/*z* 961 and 801, suggesting the presence of at least one hexose sugar and one methyl-deoxyhexose sugar in the structure of the compound.[13] Finally, a fragment ion at *m*/*z* 777 corresponding to the sodium-cationized sugar portion of compound **1** was detected, followed by species at *m*/*z* 633 and 489 generated by the elimination of one or two methyl-dideoxyhexose monosaccharides, respectively.[14] Accordingly, compound **1** could be described as a pregnane skeleton bearing two different esterified groups of molecular mass 130 and 105 Da, respectively, and an etherified sugar chain of 754 Da constituted by a terminal (nonreducing end) hexose monosaccharide linked to a methyl-deoxyhexose sugar followed by three methyl-dideoxyhexose units.

The ^1H NMR spectrum of the aglycon portion of **1** showed signals for three methyl groups at δ 1.08 (3H, s), 1.30 (3H, d, J = 6.0 Hz), and 1.64 (3H, s), for an olefinic proton signal at ca. δ 5.36 (1H, m), and for an alcoholic methine proton signal at δ 3.40 (1H, m), and two esterified signals at 4.77 (1H, dd, J = 10.0, 4.0 Hz) and 5.05 (1H, q, J = 6.0 Hz), corresponding to secondary oxygenated carbons. The ^{13}C NMR chemical shifts of all the hydrogenated carbons could be assigned unambiguously by the HSQC spectrum. In addition to the pregnane moiety, the ^1H and ^{13}C NMR spectra of compound **1** showed signals due to nicotinoyl and cinnamoyl groups (Table 1 and Experimental Section).[5] The complete elucidation of the aglycon structure of **1** was achieved by a HMBC experiment. The HMBC correlations between the proton signal at δ 1.08 (Me-19) and the carbon resonances of C-10, C-1, C-5, and C-9; the signal at δ 1.64 (Me-18) and the resonances of C-13, C-12, C-14, and C-17; the signal at δ 5.05 (H-20) and the resonances of C-16, C-13, and C-17; and the signal at δ 3.40 (H-3) and the resonances of C-1 and C-5; the signal at δ 4.77 (H-12) and the resonances of C-9, C-14, and C-17 allowed the pregnane skeleton of **1** to be established as sarcostin (Table 1).[14] It only remained to determine the relative positions at C-12 and at C-20 of the cinnamoyl and nicotinoyl residues. The long-range shift correlations between the carbonyl carbon at 168.0 ppm and H-2^{II} (δ 6.62 1H, d, J = 15.9 Hz) and H-3^{II} (δ 7.70 1H, d, J = 15.9 Hz) of the cinnamoyl moiety as well as H-12 (δ 4.77) of the pregnane moiety were observed in the HMBC spectrum. Therefore, the cinnamoyl moiety is esterified at the C-12 position. The aglycon of compound **1** was thus deduced as 12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin. In the ^1H NMR spectrum (Table 2), five anomeric proton signals (δ 4.30, 4.44, 4.74, 4.87, 4.89) and four methyl doublets (δ 1.21, 1.24, 1.26, 1.40) were observed. 2D-

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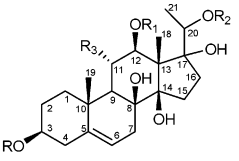
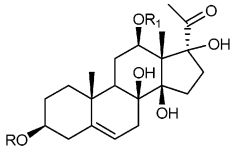
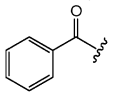


compound	R	R_1	R_2	R_3
1	A	Cinn	Nic	H
3	C	Cinn	H	H
5	D	Cinn	H	OH
7	B	Cinn	Bz	H
8	B	Cinn	H	H
9	B	Bz	Bz	H
12	G	Bz	Bz	H
14	L	Cinn	H	H
15	I	Cinn	H	H

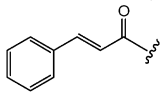


compound	R	R_1
2	F	Cinn
4	C	Cinn
6	A	Cinn
10	B	Cinn
11	E	Acetyl
13	H	Coum
16	I	Cinn
17	L	Cinn
18	M	Acetyl

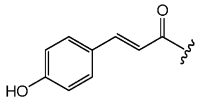
Bz = benzoyl



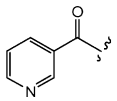
Cinn = cinnamoyl;



Coum = coumaroyl



Nic = nicotinoyl



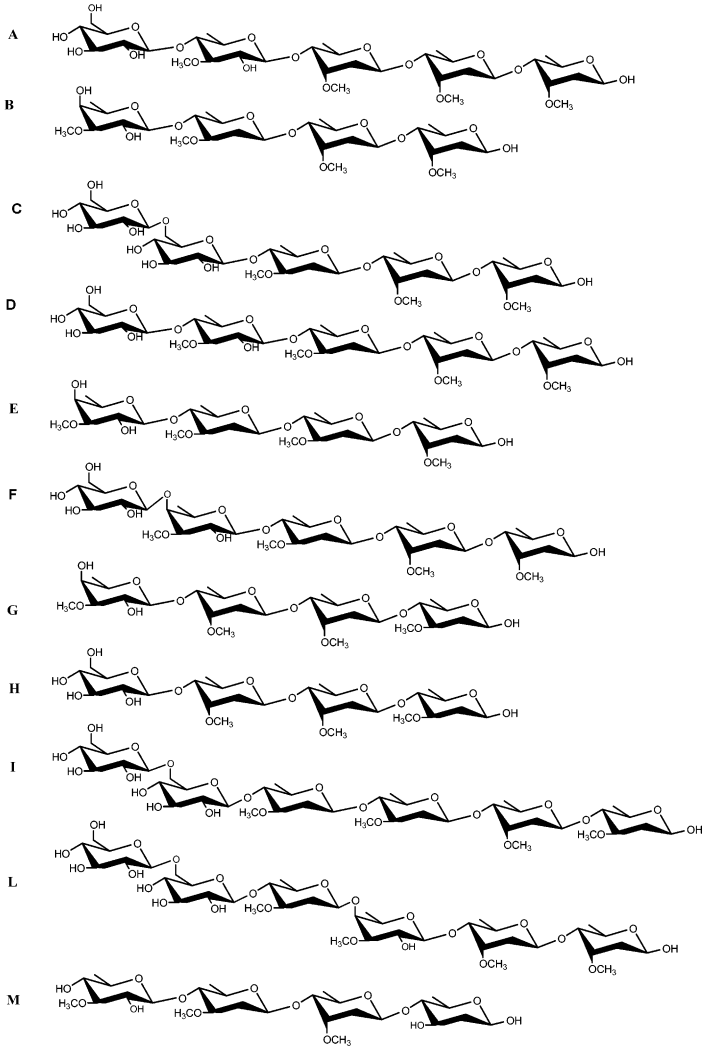


Figure 1. Structures of compounds **1**−**18**.

TOCSY experiments, together with the DQF-COSY spectra, led us to establish the proton sequence within these sugar fragments as three β-cymaropyranosyl units, one β-thevetopyranosyl unit, and one β-glucopyranosyl unit.[15] In the HSQC experiment, glycosidation shifts were observed for C-4_{cymI}, C-4_{cymII}, C-4_{cymIII}, and C-4_{the}. The positions of the sugar units were defined unambiguously by the HMBC experiments: the β-cymarose unit was linked at C-3 as shown by the cross-peak between δ 4.89 (H-1_{cymI}) and 79.0 (C-3), and key correlations were observed between H-1_{CimII}−C-4_{cymI}, H-1_{the}−C-4_{CimIII}, and H-1_{glc}−C-4_{the}. The β-linkages of the five sugar moieties were shown by the large ($J \approx 8.5$ Hz) coupling constants of the anomeric proton signals as well as by the resonances of C-2, C-3, and C-5 characteristic of β forms.[16,17] The absolute configurations of the sugar units of compounds **1**−**18** were assigned after acid hydrolysis and fractionation of the hydrolyzate by silica gel column chromatography, which afforded cymarose and thevetose. These sugars were determined to be in the D-form on the basis of their optical rotation values.[7] In the case of glucose the absolute configuration was assigned after hydrolysis of the compounds with 1 N HCl, trimethylsilation of the hydrolyzate, and GC analysis on a Chiral-Val column. The absolute configuration of glucose was established as D by comparison with retention times of authentic samples of D- and L-glucose.[8] On the basis of this NMR and chemical evidence, the structure of the sugar chain of compound **1** was determined to be β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **2** (chain F) had a molecular formula of $C_{64}H_{96}O_{25}$, as determined from its ^{13}C NMR and DEPT spectra and by ESIMS in the positive-ion mode. The full ESIMS of **2** showed the presence of a major peak at *m*/*z* 1287 and indicated a molecular weight of 1264 for this compound. Fragmentation of this sodium-cationized species generated a single fragment at *m*/*z* 1139 due to the elimination of one neutral molecule of cinnamic acid, and further fragmentation of this species produced in the MS^3 spectrum three different peaks at *m*/*z* 1079, 977, and 817; the latter two peaks were generated by the consecutive loss of one hexose sugar and one methyl-deoxyhexose sugar. The identity of the *m*/*z* 1079 fragment was deduced by a comparison with previously described data,[11] demonstrating that mass fragmentation of pregnane skeletons having a keto group at C-20 and a hydroxyl group on C-14 determines a 60 neutral loss, due to elimination of CH_3COOH. Also in this case fragments corresponding to the sodium-adducted sugar chain were observed (Experimental Section).

The ^{13}C NMR and DEPT spectra of **2** showed 64 signals, of which 21 were assigned to a pregnane aglycon, nine to a cinnamoyl residue, and 34 to the sugar units. The ^{1}H NMR spectrum of **2** showed for the aglycon moiety three methyl proton signals at δ 1.18, 1.63, and 2.24 (each s) and two methine proton signals indicative of secondary alcoholic functions at δ 3.50 (1H, brm, H-3)

Table 1. ^{13}C NMR Data for Aglycon Moieties of **1**−**3**, **5**, **7**, **9**, and **11** (CD_3OD, 600 MHz)[a]

position	**1**	**2**	**3**	**5**	**7**	**9**	**11**
1	38.6	39.6	38.6	38.8	38.6	38.6	39.6
2	30.5	30.0	30.8	31.4	30.5	30.2	30.0
3	79.0	78.7	79.3	78.7	79.0	79.0	78.1
4	38.0	39.8	38.1	38.3	38.0	38.0	39.4
5	140.0	140.0	140.0	140.3	139.9	139.9	140.0
6	120.0	119.5	119.9	120.2	119.7	119.7	119.5
7	34.5	34.7	34.5	33.0	34.4	34.5	34.7
8	75.0	74.4	75.0	75.0	75.0	75.0	74.4
9	44.5	45.0	44.7	50.0	44.6	44.7	45.0
10	37.6	37.5	38.0	36.9	38.0	37.6	37.0
11	26.1	25.2	26.1	72.0	26.0	26.0	25.2
12	76.0	74.0	76.4	78.7	76.3	76.0	75.0
13	57.6	57.7	57.3	56.0	57.2	57.7	57.7
14	87.8	89.7	88.8	86.0	88.9	88.8	89.7
15	35.2	34.0	35.1	35.0	35.0	35.2	34.0
16	34.0	33.0	34.1	33.8	33.8	34.1	33.0
17	90.0	92.0	90.0	89.8	90.0	89.8	92.0
18	11.4	10.6	11.3	11.3	11.5	11.3	10.6
19	18.6	18.3	18.3	18.2	18.3	18.6	18.3
20	74.3	210.0	71.6	74.2	74.8	74.3	210.0
21	15.5	27.7	18.7	15.8	15.3	15.5	27.9
Ac							
COO				172.8			172.3
CH_3				21.7			21.3
Cinn at C-12							
1^I	168.0	167.3	167.3		167.3		
2^I	118.9	118.6	118.6		118.6		
3^I	146.7	146.0	146.0		146.0		
4^I	135.7	135.0	135.0		135.0		
5^I	130.1	129.0	129.0		129.0		
6^I	129.2	129.8	128.8		128.8		
7^I	131.6	131.7	131.7		131.7		
8^I	129.2	129.8	128.8		128.8		
9^I	130.1	129.0	129.0		129.0		
Nic at C-20							
2^I	153.9						
3^I	127.3						
4^I	136.3						
5^I	123.3						
6^I	153.7						
COO	166.0						
Bz at C-20							
1^I	132.0				131.0	132.0	
$2^I/6^I$	129.5				130.0	129.5	
$3^I/5I$	130.8				130.3	130.8	
4^I	133.6				134.0	133.6	
COO	167.6				168.6	167.6	
Bz at C-20							
1^I						132.0	
$2^I/6^I$						129.5	
$3^I/5^I$						130.8	
4^I						133.6	
COO						167.6	

[a] Assignments were confirmed by HSQC and HMBC experiments.

and δ 4.70 (1H, dd, $J = 10.5$ and 4.0 Hz, H-12). In addition to the pregnane moiety, the ^1H NMR spectrum of compound **2** showed signals due to a *trans*-cinnamoyl residue (Table 1 and Experimental Section). The ^{13}C NMR spectrum of **2** also suggested for the aglycon moiety a pregnane skeleton with two secondary alcoholic functions (78.1 and 74.0 ppm) and three tertiary alcoholic functions (74.4, 89.7, and 92.0 ppm). On the basis of HSQC and HMBC data, the aglycon of **2** was identified as 12-*O*-cinnamoyldeacetylmetaplexigenin.[18] The ^{13}C NMR spectrum of **2** showed a sugar portion made up of three 3-*O*-methyl-2,6-dideoxyhexopyranose units, one 3-*O*-methyl-6-deoxyhexopyranose unit, and one hexopyranose unit. Five signals were assigned to anomeric carbons (δ 98.9, 100.0, 102.4, 103.2, 104.6), four to methoxyl groups (δ 57.8, 58.2, 58.3, 58.6), and four to methyl groups (18.5, 18.3, 18.6, 17.8). The structure elucidation of the sugar portion was achieved using 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments (Table 2). The isolated anomeric proton signals resonating at the uncrowded region of the spectrum (between 4.45 and 4.81 ppm) were the starting point for the 1D-TOCSY experiments. The 1D-TOCSY subspectra obtained by irradiating the anomeric proton at δ 4.45 allowed us to establish this proton as belonging to a glucose unit. In the case of the 2,6-dideoxyhexoses and 6-dideoxyhexose, an easier identification of all the proton signals was obtained by recording the 1D-TOCSY spectrum by also irradiating the methyl doublets. The identification of each proton signal in the 1D-TOCSY spectrum was deduced by a DQF-COSY experiment (Table 2). The β-configuration of the five sugar units was shown by the large coupling constants of the anomeric proton signals ($J = 8.0$ Hz for the glucose and digitalose units, and J between 8.5 and 9.5 Hz for the 2,6-dideoxyhexoses).[17,20] A HSQC experiment allowed the assignment of all the carbon resonances and therefore the identification of the sugars as a terminal β-glucopyranosyl unit (H-1 δ 4.45), one β-digitalopyranosyl unit (H-1 δ 4.48), two β-cymaropyranosyl units (H-1 δ 4.81; H-1 δ 4.72), and one β-oleandropyranosyl unit (H-1 δ 4.63). All the substituted sugars were glycosidated at C-4. The sugar sequence was deduced from the HMBC experiment, which showed cross-peaks between C-3_{agl} and H-1_{cym1}; H-1_{cym1I} and C-4 $_{cym1}$; H-1_{ole} and C-4_{cym1I}; H-1_{dig} and C-4_{ole}; and H-1_{glc} and C-$4_{dig.}$ Thus, the new compound **2** was defined as the new kidjolanin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-digitalopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **3** (chain C) was assigned a molecular formula of $C_{63}H_{96}O_{26}$ as determined from the ^{13}C NMR, DEPT, and ESIMS (positive-ion mode) data. In the ESIMS of **3**, a main signal at *m*/*z* 1291 was observed, and a molecular weight of 1268 was deduced. Also in this case, a comparison with our previous data allowed the deduction of the presence of a hydroxyl group at both C-20 and C-14[12] (see Experimental Section).

Comparison of the ^1H and ^{13}C NMR data of the aglycon of **3** with those of **1** indicated some structural similarity. The main differences were the absence of the signals for one nicotinoyl residue, the upfield shift of C-20 (δ 71.6) and downfield shift of C-21 (δ 18.7) and C-17 (δ 90.0) in the ^{13}C NMR spectrum, and the upfield shift of H-20 (δ 3.88) in the ^1H NMR spectrum, implying the absence of a nicotinoyl ester moiety at C-20. Thus, the aglycon of compound **3** was identified as 12-*O*-cinnamoylsarcostin.[5] The sugar chain attached at C-3 of the aglycon was a pentasaccharide, as revealed by five anomeric signals (δ 4.89, 4.84, 4.65, 4.62, 4.56) in its ^1H NMR spectrum. The proton coupling network of each sugar residue was derived from a combination of 1D and 2D NMR experiments, which indicated that a β-D-glucopyranose unit was present instead of the β-D-digitalopyranose observed in the saccharide chain of **2** (Tables 2 and 3). To establish the nature of the sugar sequence, an analysis of the HMBC spectrum was performed, which showed correlation peaks between H-1 of the terminal glucose unit and C-6 of the inner glucose. Thus, the structure of compound **3** was established as 12-*O*-cinnamoylsarcostin 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **4** (chain C) exhibited a molecular formula of $C_{63}H_{94}O_{26}$, as determined from the ^{13}C NMR, DEPT, and ESIMS (positive-ion mode) data. The ESIMS and ESIMS^n data obtained for compound **4** were very similar to those of compound **3**. The only observed differences were the sodiated molecular ions (*m*/*z* 1289 for **4** vs *m*/*z* 1291 for compound **3**) and the occurrence of a 60 Da neutral loss in the MS^3 spectrum of **4** instead of the 44 Da neutral loss observed for compound **3**. These results suggested that compound **3** and compound **4** differed only in the structure of the C-17 side chain. Analysis of the NMR and MS data of **4** revealed the same aglycon of compound **2** and a sugar chain made up of five monosaccharides (Table 3). Interpretation of the NMR data of compound **4** and comparison with those of **3** showed they possess

Table 2. ^{1}H and ^{13}C NMR Data for Oligosaccharide Moieties of **1**, **2**, **5**, and **6** (CD_3OD, 600 MHz)[a,b]

	1,**6** chain A		**2** chain F		**5** chain D	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
CymI						
1	4.89 dd (9.5, 2.0)	97.0	4.81 dd (9.5, 2.0)	98.9	4.89 dd (9.0, 2.0)	97.5
2a	1.54 br dd (16.0, 12.0)	36.4	1.71 br dd (16.0, 12.0)	37.6	1.54 br dd (15.0, 12.0)	36.5
2b	2.10 br dd (16.0, 3.0)		2.12 br dd (16.0, 3.0)		2.09 br dd (15.0, 3.0)	
3	3.87 q (3.0)	78.4	4.01 q (3.0)	79.5	3.87 q (3.0)	78.3
4	3.30 dd (9.5, 3.0)	84.0	3.39 dd (9.5, 3.0)	83.8	3.30 dd (9.5, 3.0)	83.5
5	3.90 dq (9.5, 6.0)	70.0	3.89 dq (9.5, 6.0)	69.8	3.90 dq (9.5, 6.0)	69.9
6	1.21 d (6.0)	18.5	1.24 d (6.0)	18.5	1.21 d (6.0)	18.4
−OMe	3.48 s	58.4	3.45 s	58.2	3.50 s	58.2
CymII						
1	4.74 dd (8.5, 1.5)	101.4	4.72 dd (9.0, 2.0)	100.0	4.84 dd (9.5, 2.0)	101.1
2	1.64 m, 2.12 m	36.4	1.62 m, 2.15 m	37.2	1.63 m, 2.13 m	36.4
3	3.89 m	78.6	3.86 m	78.1	3.88 m	78.5
4	3.24 t (9.0, 2.5)	84.0	3.34 t (9.0, 3.0)	83.5	3.25 dd (9.5, 3.0)	83.8
5	3.84 dq (9.0, 6.0)	70.2	3.80 dq (9.5, 6.2)	70.4	3.86 dq (9.5, 6.0)	70.0
6	1.26 d (6.0)	18.1	1.28 d (6.2)	18.3	1.23 d (6.0)	18.4
−OMe	3.52 s	58.3	3.48 s	58.3	3.50 s	58.3
Ole						
1			4.63 dd (9.0, 2.0)	102.4	4.65 dd (8.5, 1.5)	102.6
2a			1.47 ddd (13.0, 9.0, 9.0)	37.6	1.48 ddd (13.0, 9.0, 9.0)	37.6
2b			2.38 ddd (13.0, 4.0, 2.0)		2.35 ddd (13.0, 4.0, 2.0)	
3			3.18 ddd (9.5, 9.0, 4.0)	80.4	3.45 ddd (9.5, 9.0, 4.0)	80.3
4			3.24 dd (9.5, 9.5)	84.5	3.25 dd (9.5, 9.5)	84.2
5			3.38 dq (9.5, 6.4)	72.6	3.44 dq (9.5, 6.0)	71.8
6			1.38 d (6.4)	18.6	1.38 d (6.0)	19.0
−OMe			3.46 s	57.8	3.47 s	58.1
CymIII						
1	4.87 dd (9.0, 1.7)	101.2				
2	1.60 m, 2.10 m	36.6				
3	3.93 q (3.0)	78.8				
4	3.26 dd (9.5, 3.0)	83.8				
5	3.84 dq (9.5, 6.0)	70.0				
6	1.24 d (6.0)	18.2				
−OMe	3.50 s	58.5				
The						
1	4.30 d (7.5)	105.6			4.35 d (8.0)	105.8
2	3.34 dd (9.5, 7.5)	71.4			3.30 dd (8.0, 9.5)	71.5
3	3.22 dd (9.5, 9.5)	86.0			3.22 (9.5, 9.5)	86.2
4	3.41 dd (9.5, 9.5)	82.8			3.43 (9.5, 9.5)	82.8
5	3.56 m	72.1			3.57 m	72.3
6	1.40 d (6.5)	18.6			1.39 d (6.5)	18.7
−OMe	3.66 s	61.3			3.63 s	61.2
Dig						
1			4.48 d (8.0)	103.2		
2			3.85 dd (9.2, 8.0)	72.0		
3			3.44 dd (9.2, 3.5)	85.3		
4			3.90 (3.5, 2.0)	77.6		
5			3.65 m	71.0		
6			1.33 d (6.5)	17.8		
−OMe			3.46	58.6		
Glc						
1	4.44 d (8.0)	105.0	4.45 d (7.6)	104.6	4.38 d (7.8)	104.7
2	3.18 dd (9.5, 8.0)	75.5	3.22 dd (9.2, 7.6)	75.2	3.22 dd (9.5, 7.8)	75.3
3	3.31 t (9.5)	78.0	3.38 t (9.2)	78.0	3.38 t (9.5)	78.0
4	3.24 t (9.5)	71.4	3.24 t (9.2)	71.1	3.26 t (9.5)	71.0
5	3.27 m	78.5	3.30 m	78.3	3.32 m	78.2
6a	3.65 dd (12.0, 5.0)	63.1	3.90 dd (12.0, 2.0)	62.6	3.69 dd (12.0, 5.0)	62.5
6b	3.84 dd (12.0, 3.5)		3.72 dd (12.0, 5.0)		3.93 dd (12.0, 3.0)	

[a] Assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments. [b] δ value, J in Hz.

the same sugar chain at C-3. Therefore, the structure kidjolanin 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside was assigned to **4**.

Compound **5** (chain D) was assigned the molecular formula $C_{64}H_{98}O_{26}$, as shown by the ESIMS data (*m*/*z* 1305 $[M + Na]^+$) in combination with the ^{13}C NMR spectrum. 11α-Hydroxy-12-*β*-*O*-cinnamoylsarcostin was determined as the aglycon moiety of **5** (Table 1 and Experimental Section).[21] The structure of the sugar chain of compound **5** was elucidated on the basis of its similarity with that of **2**. Once again, the proton coupling network within each sugar residue was determined using a combination of NMR and ESIMS experiments, which led to the identification of two cymarose units and one oleandrose, one thevetose, and one glucose unit. Direct evidence for the sugar sequence and the linkage sites was derived from HSQC and HMBC experiments, which established that the pentasaccharide chain at C-3 of **5** contains a terminal *β*-D-glucopyranose, an inner *β*-D-thevetopyranose, *β*-D-oleandropyranose, and two *β*-D-cymaropyranoses. All the substituted sugars were glycosidated at C-4. Thus compound **5** was defined as 11α-hydroxy-12-*β*-*O*-cinnamoylsarcostin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-thevetopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Compound **6** (chain A) gave the molecular formula $C_{64}H_{96}O_{25}$, as deduced from the ESIMS and from NMR spectroscopic analysis. An examination of NMR spectra of this compound revealed signals

Table 3. ^{1}H and ^{13}C NMR Data for Oligosaccharide Moieties of **3**, **4**, and **7**−**11** (CD_3OD, 600 MHz)[a,b]

	3,**4** chain C		**7**−**10** chain B		**11** chain E	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
CymI						
1	4.89 dd (9.0, 2.0)	97.5	4.90 dd (9.5, 2.0)	98.0	4.92 dd (9.2, 2.0)	98.6
2	1.54 m, 2.09m	36.4	1.57 m, 2.12 m	37.5	1.57 m, 2.16 m	36.4
3	3.87 q (3.0)	78.3	3.85 q (3.0)	79.4	3.86 q (3.0)	78.4
4	3.30 dd (9.5, 3.0)	83.5	3.30 dd (9.5, 3.0)	84.2	3.29 dd (9.5, 3.0)	83.7
5	3.90 dq (9.5, 6.0)	69.9	3.88 dq (9.5, 6.0)	70.3	3.85 dq (9.5, 6.0)	70.2
6	1.21 d (6.0)	18.4	1.28 d (6.0)	18.3	1.25 d (6.0)	18.2
−OMe	3.50 s	58.2	3.48 s	57.3	3.46 s	58.3
CymII						
1	4.84 dd (9.5, 2.0)	101.1	4.82 dd (9.0, 2.0)	99.2		
2	1.63 m, 2.13 m	36.4	1.60 m, 2.18 m	37.3		
3	3.88 q (3.0)	78.5	3.85 q (3.0)	78.9		
4	3.25 dd (9.5, 3.0)	83.8	3.32 t (9.0, 3.0)	83.0		
5	3.86 dq (9.5, 6.0)	70.0	3.83 dq (9.5, 6.2)	69.5		
6	1.23 d (6.0)	18.4	1.26 d (6.2)	18.6		
−OMe	3.50 s	58.3	3.46 s	58.8		
OleI						
1					4.68 dd (9.6, 1.8)	102.7
2a					1.39 ddd (13.0, 9.6, 9.0)	38.0
2b					2.33 ddd (13.0, 4.0, 1.8)	
3					3.37 ddd (9.6, 9.0, 4.0)	80.5
4					3.15 dd (9.5, 9.5)	84.1
5					3.33 dq (9.5, 6.1)	71.7
6					1.37 d (6.1)	18.5
−OMe					3.43 s	57.7
OleII						
1	4.65 dd (8.5, 1.5)	102.6	4.56 dd (9.0, 2.0)	102.4	4.61 dd (9.6, 1.8)	102.9
2a	1.48 ddd (13.0, 9.0, 9.0)	37.6	1.46 ddd (13.0, 9.0, 9.0)	37.6	1.44 ddd (13.0, 9.6, 9.0)	38.6
2b	2.35 ddd (13.0, 4.0, 2.0)		2.37 ddd (13.0, 4.0, 2.0)		2.35 ddd (13.0, 4.0, 1.8)	
3	3.45 ddd (9.5, 9.0, 4.0)	80.3	3.31 ddd (9.5, 9.0, 4.0)	80.4	3.40 ddd (9.6, 9.0, 4.0)	80.2
4	3.25 dd (9.5, 9.5)	84.2	3.16 dd (9.5, 9.5)	84.5	3.21 dd (9.5, 9.5)	84.0
5	3.44 dq (9.5, 6.0)	71.8	3.43 dq (9.5, 6.4)	72.6	3.43 dq (9.5, 6.1)	72.2
6	1.38 d (6.0)	19.0	1.42 d (6.4)	18.6	1.39 d (6.1)	19.0
−OMe	3.47 s	58.1	3.50 s	57.8	3.46 s	57.5
Dig						
1			4.54 d (8.0)	103.9	4.50 d (8.0)	103.2
2			3.88 dd (9.2, 8.0)	70.9	3.85 dd (8.0, 9.5)	71.0
3			3.39 dd (9.3, 3.5)	85.3	3.42 dd (9.5, 3.5)	84.3
4			3.85 dd (3.5, 2.0)	69.6	3.83 dd (3.5, 2.2)	70.2
5			3.61 m	70.9	3.65 m	71.2
6			1.36 d (6.5)	16.6	1.40 d (6.5)	16.8
−OMe			3.44 s	56.8	3.48 s	58.5
GlcI						
1	4.62 d (7.6)	104.3				
2	3.18 dd (9.5, 7.6)	75.6				
3	3.38 dd (9.5, 9.5)	78.0				
4	3.33 dd (9.5, 9.5)	71.3				
5	3.45 m	77.0				
6a	3.91 dd (12.0, 5.0)	68.6				
6b	4.18 dd (12.0, 3.0)					
GlcII						
1	4.56 d (7.8)	105.0				
2	3.19 dd (9.5, 7.8)	76.0				
3	3.24 t (9.5)	78.0				
4	3.34 t (9.5)	71.1				
5	3.53 m	78.3				
6a	3.65 dd (12.0, 5.0)	62.7				
6b	3.89 dd (12.0, 3.0)					

[a] Assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments. [b] δ value, J in Hz.

of protons and carbons attributed to the aglycon 12-*O*-cinnamoyldeacetylmetaplexigenin. Data for the glycosidic chain strongly suggested that the identity of the sugar moiety of compound **6** is the same as that described for compound **1** (Table 2 and Experimental Section). Therefore, compound **6** was determined to be 12-*O*-cinnamoyldeacetylmetaplexigenin *β*-D-glucopyranosyl-(1→4)-*β*-D-thevetopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Compounds **7**−**10** (chain B) gave molecular formulas of $C_{65}H_{92}O_{21}$, $C_{58}H_{88}O_{20}$, $C_{63}H_{90}O_{21}$, and $C_{58}H_{86}O_{20}$, respectively, as deduced from elemental analysis and their ESIMS, and NMR spectra. Their aglycons were identified as 12-*β*-*O*-cinnamoyl-20-*O*-benzoylsarcostin, 12-*β*-*O*-cinnamoylsarcostin, 12,20-*β*-*O*-dibenzoylbenzoylsarcostin, and 12-*O*-cinnamoyldeacetylmetaplexigenin, respectively, from their NMR and ESIMS^n data (Table 1 and Experimental Section). In addition to the aglycon signals, ^{13}C NMR spectra of all these compounds exhibited 28 signals, ascribable to the saccharide portion made up of three 3-*O*-methyl-2,6-dideoxyhexopyranosyl units and one 3-*O*-methyl-6-deoxyhexopyranosyl unit. A detailed comparison of the sugar region NMR spectra showed that the saccharide chain was identical in these four compounds. Their NMR data, when compared with those of compound **2**, showed that sugar chain of **7**−**10** differed from **2** only in the absence of the terminal glucopyranosyl unit (Tables 2

Table 4. ^{1}H and ^{13}C NMR Data for Oligosaccharide Moieties of **12**, **13**, and **18** (CD_3OD, 600 MHz)[a,b]

	12 chain G		**13** chain H			**18** chain M	
position	δ_H	δ_C	δ_H	δ_C	position	δ_H	δ_C
Ole					Can		
1	4.70 dd (9.3, 2.0)	98.8	4.72 dd (9.3, 2.0)	98.7	1	4.76 dd (9.5, 1.6)	98.6
2a	1.43 ddd (13.0, 9.3, 9.0)	37.4	1.41 ddd (13.0, 9.3, 9.0)	37.8	2a	2.12 ddd (13.5, 9.5, 9.0)	40.8
2b	2.30 ddd (13.0, 3.5, 2.0)		2.27 ddd (13.0, 3.5, 2.0)		2b	1.44 ddd (13.5,1.6, 3.5)	
3	3.40 ddd (9.3, 9.0, 3.5)	80.2	3.39 ddd (9.3, 9.0, 3.5)	80.0	3	3.40 ddd (9.3, 9.0, 3.5)	70.7
4	3.19 dd (9.3, 9.3)	84.2	3.16 dd (9.3, 9.3)	84.1	4	2.95 dd (9.5, 9.5)	88.9
5	3.37 dq (9.3, 6.0)	72.1	3.35 dq (9.3, 6.0)	72.0	5	3.60 dq (9.5, 6.0)	71.6
6	1.29 d (6.0)	18.3	1.30 d (6.0)	18.3	6	1.27 d (6.0)	18.4
−OMe	3.43 s	57.6	3.44 s	57.5			
CymI							
1	4.89 dd (9.5, 1.5)	100.5	4.94 dd (9.2, 2.0)	99.0			
2	1.53 m, 2.18 m	36.2	1.58 m, 2.20 m	36.3			
3	3.88 q (3.0)	78.0	3.90 q (3.0)	78.4			
4	3.34 dd (9.5, 3.0)	83.5	3.29 t (9.3, 3.0)	83.9			
5	3.83 dq (9.5, 6.0)	70.2	3.85 dq (9.3, 6.2)	70.1			
6	1.28 d (6.0)	18.4	1.26 d (6.2)	18.5			
−OMe	3.46 s	58.2	3.46 s	58.4			
CymII					Cym		
1	4.85 dd (9.5, 1.5)	101.0	4.88 dd (9.5, 2.0)	101.0	1	4.78 dd (9.5, 1.5)	101.0
2	1.65 m, 2.13 m	36.5	1.66 m, 2.15 m	36.5	2	1.68 m, 2.22 m	36.8
3	3.88 q (3.0)	78.6	3.85 q (3.0)	78.6	3	3.90 brm	78.3
4	3.25 (9.5, 3.0)	84.1	3.25 (9.5, 3.0)	84.1	4	3.38 dd (9.5, 3.0)	83.8
5	3.84 (9.5, 6.0)	69.9	3.84 (9.5, 6.0)	69.9	5	3.96 dq (9.5, 6.5)	70.5
6	1.23 d (6.0)	18.0	1.25 d (6.0)	18.0	6	1.25 d (6.5)	18.2
−OMe	3.52 s	58.1	3.52 s	58.1	OMe	3.48 s	58.5
Dig					Ole		
1	4.54 d (8.0)	102.9			1	4.60 dd (9.5, 2.0)	102.5
2	3.88 dd (9.2, 8.0)	70.9			2a	1.45 ddd (13.5, 9.5, 9.2)	37.4
3	3.39 dd (9.3, 3.5)	85.3			2b	2.34 ddd (13.5, 4.0, 2.0)	
4	3.85 dd (3.5, 2.0)	69.6			3	3.40 ddd (9.5, 9.2, 4.0)	80.2
5	3.61 m	70.9			4	3.23 dd (9.5, 9.5)	84.2
6	1.36 d (6.5)	16.6			5	3.41 dq (9.5, 6.5)	72.4
−OMe	3.44 s	56.8			6	1.37 d (6.0)	18.8
					OMe	3.45 s	57.7
Glc					The		
1			4.44 d (8.0)	104.4	1	4.44 d (7.9)	106.3
2			3.23 dd (9.5, 8.0)	75.3	2	3.31 dd (7.9, 9.5)	72.8
3			3.35 dd (9.5, 9.5)	77.8	3	3.25 dd (9.5, 9.5)	86.7
4			3.47 dd (9.5, 9.5)	71.5	4	3.49 dd (9.5, 9.5)	72.0
5			3.48 m	77.6	5	3.54 m	73.4
6a			3.71 dd (12.0, 5.0)	62.5	6	1.40 d (6.5)	18.8
6b			3.89 dd (12.0, 3.0)		OMe	3.66 s	61.4

[a] Assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments. [b] δ value, J in Hz.

and 3). These results were also confirmed by MS data: in the fragmentation of all four compounds the same fragment at *m*/*z* 615, corresponding to the sodium adduct ion of the whole tetrasaccharide chain, was detected, and a further fragmentation of these ions then generated the two main product ions at *m*/*z* 471 and 327. The D-configuration of the sugar units was obtained as reported for compound **1**. Therefore, the structure 3-*O*-β-D-digitalopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside was assigned to the sugar chain of compounds **7**−**10**.

Compound **11** (chain E) was assigned the molecular formula $C_{51}H_{82}O_{20}$, as deduced from elemental analysis, ESIMS, and the NMR spectra. The aglycon was identified as metaplexigenin by NMR data (Table 1 and Experimental Section).[5] The structure metaplexigenin 3-*O*-β-D-digitalopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside was assigned to glycoside **11** because the sugar chains of compound **11** and compounds **7**−**10** were shown to be isomers with identical fragmentation pattern in the ESIMS (see Experimental Section for details).

However, NMR spectra of the sugar chain of **11** were very close to those of **7**−**10** except that the signals assigned to one cymaropyranose unit were missing in **11** and replaced by seven signals ascribable to a C-4 glycosylated oleandrose. To establish the nature of the sugar sequence, supported by the analysis of the fragmentation pattern in the ESIMS, an analysis of the ROESY spectrum was achieved, which showed correlation peaks between H-4 of oleandrose I and H-1 of oleandrose II and between H-1 of digitalose and H-4 of oleandrose II, respectively.

Compound **12** (chain G) gave a molecular formula of $C_{63}H_{90}O_{21}$, as deduced from elemental analysis and its ESIMS and NMR spectra. The aglycon was identified as 12,20-β-*O*-dibenzoylsarcostin by NMR data (Table 1 and Experimental Section).[5] In addition to the aglycon signals, the ^{13}C NMR spectrum exhibited 28 signals ascribable to the saccharide portion, made up of three 3-*O*-methyl-2,6-dideoxyhexopyranosyl units and one 3-*O*-methyl-6-deoxyhexopyranosyl unit. In the ^{1}H NMR spectrum (Table 4), four anomeric proton signals (δ 4.89, 4.85, 4.70, 4.54) and four methyl doublets (δ 1.36, 1.29, 1.28, 1.23) were observed. 2D-TOCSY NMR experiments, together with the DQF-COSY spectra, led us to establish the proton sequence within these sugar fragments as two β-cymaropyranosyl units, one β-oleandropyranosyl unit, and one β-digitalopyranosyl unit. In a HSQC experiment, glycosidation shifts were observed for C-4_{cymI}, C-4_{cymII}, and C-4_{ole}. The positions of the sugar units were defined unambiguously by a HMBC experiment: the β-oleandrose unit was linked at C-3 as shown by the cross-peak between δ 4.70 (H-1_{ole}) and 79.0 (C-3); key correlations were observed between H-1_{cymI}−C-4_{ole}, H-1_{cymII}−C-4_{cymI}, and H-1_{dig}−C-4_{cymII}. On the basis of this NMR evidence, the structure of the sugar chain of compound **12** was determined to be β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside.

Table 5. ^{1}H and ^{13}C NMR Data for Oligosaccharide Moieties of **14**−**17** (600 MHz, CD_3OD)[a,b]

	14,**17** chain L			**15**,**16** chain M	
position	δ_H	δ_C	position	δ_H	δ_C
CymI			OleI		
1	4.89 dd (9.0, 2.0)	97.2	1	4.70 dd (9.5, 1.8)	98.8
2a	1.55 br dd (16.0, 12.0)	36.2	2a	1.40 ddd (13.0, 9.5, 9.3)	38.0
2b	2.08 br dd (16.0, 3.0)		2b	2.25 ddd (13.0, 4.0, 2.0)	
3	3.89 q (3.0)	78.5	3	3.39 ddd (9.5, 9.3, 4.0)	80.2
4	3.27 dd (9.5, 3.0)	83.8	4	3.16 dd (9.5, 9.5)	84.0
5	3.86 dq (9.5, 6.0)	69.6	5	3.36 dq (9.5, 6.5)	72.1
6	1.24 d (6.0)	18.4	6	1.32 d (6.5)	18.2
−OMe	3.45 s	58.2	−OMe	3.44 s	57.2
CymII			Cym		
1	4.85 dd (9.0, 2.0)	101.0	1	4.94 dd (9.2, 2.0)	100.5
2	1.65 m, 2.17 m	36.5	2	1.55 m, 2.16 m	36.3
3	3.85 q (3.0)	78.4	3	3.86 q (3.0)	78.1
4	3.30 dd (9.5, 3.0)	84.1	4	3.28 dd (9.5, 3.0)	83.5
5	3.81 dq (9.5, 6.0)	70.0	5	3.83 dq (9.5, 6.1)	70.3
6	1.28 d (6.0)	18.6	6	1.26 d (6.1)	18.0
−OMe	3.47 s	58.2	−OMe	3.48 s	58.5
Dig			OleII		
1	4.48 d (8.0)	103.3	1	4.62 dd (9.5, 1.8)	102.3
2	3.92 dd (9.2, 8.0)	71.5	2a	1.46 ddd (13.0, 9.5, 9.3)	37.7
3	3.42 dd (9.2, 3.5)	85.3	2b	2.46 ddd (13.0, 4.0, 2.0)	
4	3.86 dd (3.5, 2.0)	77.2	3	3.41 ddd (9.5, 9.3, 4.0)	80.1
5	3.62 m	70.6	4	3.23 dd (9.5, 9.5)	84.2
6	1.30 d (6.5)	18.0	5	3.42 dq (9.5, 6.5)	72.2
−OMe	3.50	59.3	6	1.40 d (6.5)	18.5
Ole			−OMe	3.44 s	57.3
1	4.66 dd (9.4, 2.0)	102.6	OleIII		
2a	1.48 ddd (13.0, 9.4, 9.0)	37.8	1	4.64 dd (9.5, 1.8)	103.0
2b	2.36 ddd (13.0, 4.0, 2.0)		2a	1.52 ddd (13.0, 9.5, 9.3)	39.5
3	3.48 ddd (9.5, 9.4, 4.0)	80.6	2b	2.44 ddd (13.0, 4.0, 2.0)	
4	3.23 dd (9.5, 9.5)	84.5	3	3.32 ddd (9.5, 9.3, 4.0)	80.2
5	3.42 dq (9.5, 6.4)	72.0	4	3.18 dd (9.5, 9.5)	84.2
6	1.40 d (6.4)	19.0	5	3.50 dq (9.5, 6.5)	72.4
−OMe	3.44 s	57.9	6	1.34 d (6.5)	18.7
GlcI			−OMe	3.48 s	57.5
1	4.62 d (8.0)	104.0	GlcI		
2	3.21 dd (9.5, 8.0)	75.0	1	4.66 d (8.0)	104.1
3	3.33 dd (9.5, 9.5)	77.8	2	3.20 dd (9.5, 8.0)	74.9
4	3.27 dd (9.5, 9.5)	71.4	3	3.38 dd (9.5, 9.5)	77.6
5	3.45 m	77.0	4	3.33 dd (9.5, 9.5)	71.4
6a	3.86 dd (12.0, 5.0)	69.0	5	3.44 m	77.1
6b	4.14 dd (12.0, 3.5)		6a	3.89 dd (12.0, 5.0)	68.8
GlcII			6b	4.18 dd (12.0, 3.5)	
1	4.56 d (7.5)	104.3	GlcII		
2	3.23 dd (9.5, 7.5)	75.9	1	4.54 d (7.8)	104.6
3	3.25 dd (9.5, 9.5)	77.8	2	3.19 dd (9.5, 7.8)	76.0
4	3.33 dd (9.5, 9.5)	71.2	3	3.24 dd (9.5, 9.5)	77.7
5	3.52 m	78.1	4	3.29 dd (9.5, 9.5)	71.2
6a	3.67 dd (12.0, 5.0)	62.3	5	3.52 m	78.0
6b	3.88 dd (12.0, 3.0)		6a	3.70 dd (12.0, 5.0)	62.5
			6b	3.90 dd (12.0, 3.0)	

[a] Assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments. [b] δ value, J in Hz.

ESIMS and NMR data of compound **13** (chain H) indicated that it is a derivative of the C/D-*cis*-polyhydroxypregnane metaplexigenin, by comparison with the data of the aglycon of compound **2**. In addition to the pregnane moiety, the ^{1}H and ^{13}C NMR spectra of compound **13** showed signals due to a *p*-coumaroyl group. The *p*-coumaroyl moiety was located at C-12 on the basis of the chemical shift of the double doublet of H-12 (δ ~4.65 when esterified, δ ~3.50 when free, Experimental Section). This was confirmed by the results of a HMBC experiment, which showed long-range correlation peaks between the carbonyl carbon of the *p*-coumaroyl group and H-12. The sugar chain attached to the C-3 position was assigned as a tetrasaccharide, as revealed by four anomeric signals (Table 4). The proton coupling network of each sugar residue was derived from a combination of 1D and 2D NMR experiments, with a terminal β-D-glucopyranosyl unit present instead of a terminal β-D-digitalopyranosyl unit observed in the saccharide chain G of compound **12**. Direct evidence for the sugar sequence and their linkage sites was derived from the results of a HMBC experiment. On the basis of these spectroscopic data, the structure of the sugar chain of compound **13** was determined to be β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside.

Compounds **15** ($C_{70}H_{108}O_{29}$) (chain I) and **16** ($C_{70}H_{106}O_{29}$) (chain I) were a 12-β-*O*-cinnamoylsarcostin and a 12-β-*O*-cinnamoyldeacetylmetaplexigenin derivative, respectively.

Analysis of the sugar chain NMR data of compounds **15** and **16** confirmed that their saccharide chains were identical. The structures of the oligosaccharide moiety of **15** and **16** were deduced using ESIMS, 1D-TOCSY, and DQF-COSY experiments (Table 5). Thus, the chemical shifts of the sugar resonances were attributable to three β-oleandropyranosyl units ($\delta_{H-1oleI}$ = 4.70, $\delta_{H-1oleII}$ = 4.62, $\delta_{H-1oleIII}$ = 4.64,), two β-glucopyranosyl units ($\delta_{H-1glcI}$ = 4.54, $\delta_{H-1glcII}$ = 4.66), and one cymaropyranosyl unit (δ_{H-1cym} = 4.94). The absence of any glycosidation shift for one β-glucopyranose suggested that this sugar is the terminal unit. Glycosidation shifts were observed for C-4_{oleI} (84.0 ppm), C-4_{cym} (83.5 ppm), C4_{oleII} (84.2 ppm), C-4_{oleIII}

(84.1 ppm), and C-6_{glcI} (68.8 ppm). A cross-peak due to a long-range correlation (HMBC spectrum) between C-3 and H-1_{oleI} indicated that oleandrose is the residue linked at C-3 of the aglycon. In turn, cross-peak between C-4_{oleI} and H-1_{cym} suggested that cymarose is the second unit, a cross-peak between C-4_{cym} and H-1_{oleII} indicated that oleandrose is the third unit, and a cross-peak between C-4_{oleII} and H-1_{oleIII} showed that oleandrose is the fourth unit of the hexasaccharide chain. Therefore, the structure *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranoside was assigned to the sugar chain I.

Compounds **14** and **17** (chain L) were assigned molecular formulas of $C_{70}H_{108}O_{30}$ and $C_{70}H_{106}O_{30}$, respectively, as deduced from ESIMS and NMR analysis. Their spectroscopic data revealed that the aglycon moieties of compounds were 12-*β*-*O*-cinnamoylsarcostin and 12-*β*-*O*-cinnamoyldeacethylmetaplexigenin, respectively, and that they have an identical hexasaccharide sugar chain made up of two *β*-cymarose units, one *β*-digitalose unit, one *β*-oleandrose unit, and two *β*-glucose units. ESIMS^n results indicated the presence of a six-sugar chain (fragment ion at *m*/*z* 939); fragmentation of the glycosidic chain led to the observation of fragments at *m*/*z* 795, 651, and 491, whereas two consecutive 162 Da neutral losses from the intact compounds were observed in their MS^3 spectra. These results suggested a saccharide chain sequence of two hexoses followed by a methyl-dideoxyhexose unit linked to two consecutive methyl-dideoxyhexose sugars. From their NMR spectroscopic data, each carbon and proton signal was assigned, as shown in Table 5. Consequently, it was possible to identify a terminal *β*-glucopyranose as well as one inner *β*-D-glucopyranose glycosylated at C-6, an inner *β*-D-oleandropyranose, an inner *β*-D-digitalopyranose, and two *β*-D-cymaropyranoses each glycosylated at C-4, as inferred by the HSQC and HMBC spectra. The position of each sugar residue was confirmed by a 1D-ROESY experiment, which showed a cross-peak between the signal at *δ* 4.89 (H-1_{cymI}) and the signal at *δ* 3.49 (H-3), and other key correlation peaks between the signals at *δ* 4.85 (H-1_{cymII}) and 3.27 (H-4_{cymI}), *δ* 4.48 (H-1_{dig}) and 3.30 (H-4_{cym}), *δ* 4.66 (H-1_{ole}) and 3.86 (H-4_{dig}), and *δ* 4.14 (H-$6b_{glcII}$) and 4.56 (H-1_{glcII}). On the basis of the above data, the structure of the hexasaccharide chain of compounds **14** and **17** was deduced as *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitalopyranosyl-(1→4)-*β*- D-cymaropyranosyl-(1→4)-*β*- D-cymaropyranoside.

The aglycon moiety of compound **18** ($C_{50}H_{80}O_{20}$; chain M) was determined to be the same that of compound **11**. Moreover the sugar chain showed in the ^1H NMR spectrum signals corresponding to three methyls at *δ* 1.27, 1.25, and 1.37, three methoxy groups at *δ* 3.48, 3.45, and 3.66, and signals for four anomeric protons at *δ* 4.76, 4.78, and 4.60 each dd (J = 9.5, 2.0 Hz) and 4.64 d (J = 7.9 Hz) (Table 4). All of these data indicated that **18** has four sugars, with three of them being 2,6-dideoxy-hexopyranose units and one a 6-deoxy-hexopyranose unit. ESIMS, 1D-TOCSY, DQF-COSY, and HSQC experiments showed the presence of one *β*-canaropyranosyl unit, one *β*-cymaropyranosyl unit, one *β*-oleandropyranosyl unit, and one *β*-allopyranosyl unit.[22] An unambiguous determination of the sequence and linkage sites was obtained from the HMBC correlations, which allowed the deduction of the sugar sequence as 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-canaropyranoside.

The antiproliferative activity of all compounds was evaluated against the J774.A1, HEK-293, and WEHI-164 cell lines. The results are shown in Table 6. As can be seen from the results, compounds having sarcostin as aglycon possessed the weakest activity, irrespective of the ester and sugar moiety. Compounds having deacetylmetaplexigenin as the aglycon were the most active constituents. On the basis of the data in Table 6, it was possible to demonstrate that the presence of an aromatic group in C-12 of deacetylmetaplexigenin significantly increases the cytotoxic effect, while the presence of an acetyl group in place of an aromatic group restores the proliferation of the cells. Generally, cytotoxic effects of compounds **1**−**18** were slightly dependent on the number of sugar units: the ones having fewer sugar moieties exhibited more potent activity as compared with those having more sugar moieties. The results obtained confirmed the cytotoxic activity of pregnane glycosides, comparable to other similar derivatives reported in the literature.[23−25]

Table 6. In Vitro Antiproliferative Activity of Compounds **1**−**18**[a]

	cell line (IC_{50} *μ*M)		
compound	J774.A1[b]	HEK-293[c]	WEHI-164[d]
1	nd[e]	1.37 ± 0.034	0.64 ± 0.002
2	0.056 ± 0.003	0.23 ± 0.002	0.39 ± 0.001
3	0.91 ± 0.035	nd	0.94 ± 0.034
4	0.064 ± 0.002	0.35 ± 0.004	0.38 ± 0.003
5	0.78 ± 0.061	nd	nd
6	0.050 ± 0.004	0.38 ± 0.003	0.85 ± 0.008
7	1.22 ± 0.051	nd	nd
8	2.11 ± 0.022	0.46 ± 0.012	nd
9	1.86 ± 0.012	0.62 ± 0.004	nd
10	0.126 ± 0.007	0.07 ± 0.002	0.14 ± 0.001
11	0.79 ± 0.035	nd	0.72 ± 0.007
12	1.21 ± 0.024	1.78 ± 0.03	nd
13	0.12 ± 0.008	0.10 ± 0.011	0.08 ± 0.002
14	0.88 ± 0.001	nd	nd
15	nd	nd	nd
16	0.74 ± 0.022	0.43 ± 0.004	0.66 ± 0.004
17	0.62 ± 0.011	0.59 ± 0.008	0.95 ± 0.023
18	0.78 ± 0.021	nd	0.64 ± 0.005
6-MP[f]	0.003 ± 0.005	0.007 ± 0.004	0.015 ± 0.006

[a] The IC_{50} value is the concentration of compound that affords 50% reduction in cell growth (after a 3-day incubation). [b] J774.A1 = murine monocyte/macrophage cell line. [c] HEK-293 = human epithelial kidney cell line. [d] WEHI-164 = murine fibrosarcoma cell line. [e] nd = not determined. [f] 6-MP = 6-mercaptopurine.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (time proportional phase increment) used to achieved frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, HMBC, and ROESY experiments. The spectra were acquired at 600 MHz. The NMR data were processed on a Silicon Graphic Indigo2 workstation using UXNMR software. ESIMS (positive mode) were obtained using a Finnigan LC-Q Advantage Thermoquest spectrometer (San Jose, CA) equipped with Xcalibur software. GC analysis was performed on a ThermoFinnigan Trace GC apparatus using a 1-Chirasil-Val column (0.32 × 25 m). Elemental analysis was carried out using a Carlo Erba 1106 elemental analyzer. Medium-pressure liquid chromatography (MPLC) separations were conducted on a Buchi 688 system. Column chromatography was performed over Sephadex LH-20 (Pharmacia), and HPLC separations were conducted on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters C_{18} *μ*-Bondapak column (30 cm × 7.8 mm), and U6K injector. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck), and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. The whole plant of *Leptadenia pyrotechnica* (Forsk.) Decne was collected in the Dogon region, Mali, in April 2002 and identified by Mr. Amey Tapily. A voucher specimen (number DTM-220) has been deposited at the Herbarium of the Departement de Medicine Traditionelle, Bamako, Mali.

Extraction and Isolation. The dried whole plant (600 g) of *L. pyrotechnica* was defatted with *n*-hexane and then extracted with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH to give residues of 30.0, 36.2, and

71.6 g, respectively. Part of the chloroform−methanol (9:1) extract (10.0 g) was submitted to column chromatography over Sephadex LH-20 (100 × 5 cm) using MeOH as mobile phase. Ninety-two fractions (20 mL) were obtained. The fractions containing pregnane glycosides (fractions 18−28, 4.15 g) were chromatographed by MPLC on silica gel with a gradient (flow rate 2.5 mL/min) of $CHCl_3$−MeOH (from 100 to 88:12 stepwise) as eluent to afford 1270 fractions (20 mL) monitored by TLC. Fractions 180−308 (412 mg) were further purified on RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH−H_2O (38:12), to yield compounds **9** (87.5 mg, t_R = 10 min), **10** (90.0 mg, t_R = 12 min), **7** (61.5 mg, t_R = 18 min), and **12** (67 mg, t_R = 20 min). Fractions 332−495 (551 mg) were submitted to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH−H_2O (4:1) to give compounds **1** (40 mg, t_R = 28 min), **6** (60 mg, t_R = 26 min), **8** (58.4 mg, t_R = 30 min), **11** (57.5 mg, t_R = 34 min), **13** (103 mg, t_R = 38 min), and **18** (75 mg, t_R = 32 min). Fractions 532−765 (516 mg) were purified by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH−H_2O (34:16) to obtain compounds **2** (62.5 mg, t_R =11 min), **3** (57.5 mg, t_R = 9 min), **4** (76.0 mg, t_R = 12 min), and **5** (93 mg, t_R = 13 min). Finally, fractions 976−1029 (255 mg) were submitted to final separation by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) with MeOH−H_2O (65:35) to yield compounds **15** (58.5 mg, t_R = 25 min), **16** (8.0 mg, t_R = 26 min), **14** (63.0 mg, t_R = 31 min), and **17** (5 mg, t_R = 32 min).

Compound 1: amorphous powder; $[\alpha]_D^{25}$ +131.5 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.08 (3H, s, Me-19), 1.30 (3H, d, J = 6.0 Hz, Me-21), 1.64 (3H, s, Me-18), 3.40 (1H, brm, H-3), 4.77 (1H, dd, J = 10.0, 4.0 Hz, H-12), 5.05 (1H, q, J = 6.0 Hz, H-20), 5.36 (1H, m, H-6), 6.62 (1H, d, J = 15.9 Hz, H-2^{II}), 7.48 (2H, dd, J = 7.8, 1.8 Hz, H-5^{II}, H-9^{II}), 7.70 (1H, d, J = 15.9 Hz, H-3^{II}), 7.60 (2H, t, J = 7.6, 1.8 Hz, H-6^{II}, H-8^{II}), 7.62 (1H, t, J = 7.6, Hz, H-7^{II}), 7.64 (1H, t, J = 7.5 Hz, Me-5^{I}), 8.45 (1H, dd, J = 7.5, 1.5 Hz, H-6^{I}), 8.81 (1H, d, J = 7.0 Hz, H-4^{I}), 9.26 (1H, d, J = 1.5 Hz, H-2^{I}); ^{13}C NMR data of the aglycon, Table 1; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 2; ESIMS *m*/*z* 1394 $[M + Na]^+$, 1246 $[M - 148 + Na]^+$, 1123 $[M - 148 - 123 + Na]^+$, 961 $[M - 148 - 123 - 162 + Na]^+$, 801 $[M - 148 - 123 - 162 - 160 + Na]^+$, 777 $[M - 617 + Na]^+$, 633 $[M - 617 - 144 + Na]^+$, 489 $[M - 617 - 144 - 144 + Na]^+$; *anal*. C 61.23%, H 7.44%, N 1.03% O 30.33%, calcd for $C_{70}H_{101}NO_{26}$ C 61.25%, H 7.42%, N 1.02%, O 30.31%.

Compound 2: amorphous powder; $[\alpha]_D^{25}$ +81 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.18 (3H, s, Me-19), 1.63 (3H, s, Me-18), 2.24 (3H, s, Me-21), 3.50 (1H, brm H-3), 4.70 (1H, dd, J = 10.5 and 4.0 Hz, H-12), 5.38 (1H, m, H-6), 6.43 (1H, d, J = 16.0 z, H-2^{I}), 7.44 (3H overlapped, H-6^{I}, H-7^{I}, H-8^{I}), 7.62 (1H, d, J = 16.0 Hz, H-3^{I}), 7.65 (2H overlapped, H-5 ^{I}, H-9 ^{I}); ^{13}C NMR data of the aglycon, Table 1; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 2; ESIMS *m*/*z* 1287 $[M + Na]^+$, 1139 $[M - 148 + Na]^+$, 1079 $[M - 148 - 60 + Na]^+$, 977 $[M - 148 - 162 + Na]^+$, 817 $[M - 148 - 162 - 160 + Na]^+$, 777 $[M - 510 + Na]^+$, 633 $[M - 510 - 144 + Na]^+$, 489 $[M - 510 - 144 - 144 + Na]^+$, 345 $[M - 510 - 144 - 144 - 144 + Na]^+$; *anal*. C 60.73%, H 7.67%, O 31.64%, calcd for $C_{64}H_{96}O_{25}$ C 60.74%, H 7.65%, O 31.61%.

Compound 3: amorphous powder; $[\alpha]_D^{25}$ +128 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.12 (3H, d, J = 6.0 Hz, Me-21), 1.20 (3H, s, Me-19), 1.62 (3H, s, Me-18), 3.41 (1H, m, H-3), 3.88 (1H, q, J = 6.5 Hz, H-20), 4.70 (1H, dd, J = 10.0, 4.0 Hz, H-12), 5.36 (1H, m, H-6), 6.67 (1H, d, J = 16.0 Hz, H-2^{II}), 7.81(1H, t, J = 16.0, Hz, H-3^{II}), 7.42 (2H, dd, J = 8.0 and 1.5 Hz, H-5′, H-9 ^{I}), 7.63 (2H, t, J = 8.0 Hz, H-6^{I}, H-8^{I}), 7.36 (1H, t, J = 8.0 Hz, H-7^{I}); ^{13}C NMR data of the aglycon, Table 1; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1291 $[M + Na]^+$, 1143 $[M - 148 + Na]^+$, 1099 $[M - 148 - 44 + Na]^+$, 937 $[M - 148 - 44 - 162 + Na]^+$, 775 $[M - 148 - 44 - 162-162 + Na]^+$, 631 $[M - 148 - 162 - 162 - 144 + Na]^+$, 779 $[M - 512 + Na]^+$, 635 $[M - 512 - 144 + Na]^+$, 491 $[M - 512 - 144 - 144 + Na]^+$; *anal*. C 59.59%, H 7.63%, O 32.76%, calcd for $C_{63}H_{96}O_{26}$ C 59.61%, H 7.62%, O 32.77%.

Compound 4: amorphous powder; $[\alpha]_D^{25}$ −27 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **2**; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1289 $[M + Na]^+$, 1141 $[M - 148 + Na]^+$, 1081 $[M - 148 - 60 + Na]^+$, 979 $[M - 148 - 162 + Na]^+$, 817 $[M - 148 - 162 - 162 + Na]^+$, 673 $[M - 148 - 162 - 162 - 144 + Na]^+$, 779 $[M - 510 + Na]^+$, 635 $[M - 510 - 144 + Na]^+$, 491 $[M - 510 - 144 - 144 + Na]^+$; *anal*. C 59.69%, H 7.49%, O 32.84%, calcd for $C_{63}H_{94}O_{26}$ C 59.70%, H 7.48%, O 32.82%.

Compound 5: amorphous powder; $[\alpha]_D^{25}$ +22 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.12 (3H, d, J = 6.0 Hz, Me-21), 1.20 (3H, s, Me-19), 1.62 (3H, s, Me-18), 3.46 (1H, m, H-3), 3.83 (1H, q, J = 6.5 Hz, H-20),5.35 (1H, m, H-6), 4.05 (1H, t, J = 10.0 Hz H-11β), 4.85 (1H, dd, J = 10.0, 4.0 Hz, H-12α), 6.67 (1H, d, J = 16.0 Hz, H-2^{II}), 7.81(1H, t, J = 16.0, Hz, H-3^{II}), 7.42 (2H, dd, J = 8.0 and 1.5 Hz, H-5^{I}, H-9^{I}), 7.63 (2H, t, J = 8.0 Hz, H-6^{I}, H-8^{I}), 7.36 (1H, t, J = 8.0 Hz, H-7^{I}); ^{13}C NMR data of the aglycon moiety, Table 1; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 2; ESIMS *m*/*z* 1305 $[M + Na]^+$, 1157 $[M - 148 + Na]^+$, 1113 $[M - 148 - 44 + Na]^+$, 951 $[M - 148 - 44 - 162 + Na]^+$, 791 $[M - 148 - 44 - 162 - 160 + Na]^+$, 777 $[M - 528 + Na]^+$, 633 $[M - 528 - 144 + Na]^+$, 489 $[M - 528 - 144 - 144 + Na]^+$; *anal*. C 59.88%, H 7.72%, O 32.42%, calcd for $C_{64}H_{98}O_{26}$ C 59.89%, H 7.70%, O 32.41%.

Compound 6: amorphous powder; $[\alpha]_D^{25}$ +37 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **2**; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 2; ESIMS *m*/*z* 1287 $[M + Na]^+$, 1139 $[M - 148 + Na]^+$, 1079 $[M - 148 - 60 + Na]^+$, 977 $[M - 148 - 162 + Na]^+$, 817 $[M - 148 - 162 - 160 + Na]^+$, 673 $[M - 148 - 162 - 160 - 144 + Na]^+$, 777 $[M - 510 + Na]^+$, 633 $[M - 510 - 144 + Na]^+$, 489 $[M - 510 - 144 - 144 + Na]^+$, 345 $[M - 510 - 144 - 144 - 144 + Na]^+$; *anal*. C 60.73%, H 7.68%, O 31.62%, calcd for $C_{64}H_{96}O_{25}$ C 60.74%, H 7.65%, O 31.61%.

Compound 7: amorphous powder; $[\alpha]_D^{25}$ +89 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.10 (3H, s, Me-19), 1.34 (3H, d, J = 6.5 Hz, Me-21), 1.70 (3H, s, Me-18), 3.43 (1H, m, H-3), 4.76 (1H, dd, J = 10.0, 4.0 Hz, H-12), 4.98 (1H, q, J = 6.5 Hz, H-20), 5.36 (1H, m, H-6), 6.64 (1H, d, J = 16.0 Hz, H-2^{I}), 7.81(1H, t, J = 16.0, Hz, H-3^{I}), 7.44 (2H, dd, J = 8.0 and 1.5 Hz, H-5^{I}, H-9^{I}), 7.60 (2H, t, J = 8.0 Hz, H-6^{I}, H-8^{I}), 7.48 (1H, t, J = 8.0 Hz, H-7^{I}), 7.54 (2H, t, J = 7.5 Hz, H-3^{II} and H-5^{II}), 7.65 (1H, t, J = 7.5 Hz, H-4^{II}), 8.07 (2H, dd, J = 7.5, 1.5 Hz, H-2^{II} and H-6^{II}); ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1231 $[M + Na]^+$, 1083 $[M - 148 + Na]^+$, 961 $[M - 148 - 122 + Na]^+$, 801 $[M - 148 - 122 - 160 + Na]^+$, 657 $[M - 148 - 122 - 160 - 144 + Na]^+$, 615 $[M - 616 + Na]^+$, 471 $[M - 616 - 144 + Na]^+$, 327 $[M - 616 - 144 - 144 + Na]^+$; *anal*. C 64.53%, H 7.68%, O 27.79%, calcd for $C_{65}H_{92}O_{21}$ C 64.55%, H 7.67%, O 27.78%.

Compound 8: amorphous powder; $[\alpha]_D^{25}$ +76 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **3**; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1127 $[M + Na]^+$, 979 $[M - 148 + Na]^+$, 935 $[M - 148 - 44 + Na]^+$, 775 $[M - 148 - 44 - 160 + Na]^+$, 631 $[M - 148 - 44 - 160 - 144 + Na]^+$, 615 $[M - 512 + Na]^+$, 471 $[M - 512 - 144 + Na]^+$, 327 $[M - 512 - 144 - 144 + Na]^+$; *anal*. C 63.01%, H 8.05%, O 28.96%, calcd for $C_{58}H_{88}O_{20}$ C 63.03%, H 8.02%, O 28.95%.

Compound 9: amorphous powder; $[\alpha]_D^{25}$ +58 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.08 (3H, s, Me-19), 1.31 (3H, d, J = 6.5 Hz, Me-21), 1.66 (3H, s, Me-18), 3.43 (1H, m, H-3), 4.78 (1H, dd, J = 10.0, 4.0 Hz, H-12), 5.02 (1H, q, J = 6.5 Hz, H-20), 5.35 (1H, m, H-6), 7.42 (4H, t, J = 7.6 Hz, H-4^{I}, H-6^{I}, H-4^{I}, H-6^{II}), 7.59 (2H, t, J = 8.0 Hz, H-5^{I}, H-5^{II}), 8.01 (4H, dd, J = 7.5, 1.5 Hz, H-3^{I}, H-7^{I}, H-3^{II}, H-7^{II}); ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1205 $[M + Na]^+$, 1083 $[M - 122 + Na]^+$, 961 $[M - 122 - 122 + Na]^+$, 801 $[M - 122 - 122 - 160 + Na]^+$, 657 $[M - 122 - 122 - 160 - 144 + Na]^+$, 615 $[M - 590 + Na]^+$, 471 $[M - 590 - 144 + Na]^+$, 327 $[M - 590 - 144 - 144 + Na]^+$; *anal*. C 63.92%, H 7.68%, O 28.40%, calcd for $C_{63}H_{90}O_{21}$ C 63.94%, H 7.67%, O 28.39%.

Compound 10: amorphous powder; $[\alpha]_D^{25}$ +67 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **2**; ^{1}H and ^{13}C NMR data of the sugar moiety, Table 3; ESIMS *m*/*z* 1125 $[M + Na]^+$, 977 $[M - 148 + Na]^+$, 917 $[M - 148 - 60 + Na]^+$, 817 $[M - 148 - 160 + Na]^+$, 673 $[M - 148 - 160 - 144 + Na]^+$, 615 $[M - 510 + Na]^+$, 471 $[M - 510 - 144 + Na]^+$, 327 $[M - 510 - 144 - 144 + Na]^+$; *anal*. C 63.12%, H 7.87%, O 29.02%, calcd for $C_{58}H_{86}O_{20}$ C 63.14%, H 7.86%, O 29.00%.

Compound 11: amorphous powder; $[\alpha]_D^{25}$ +135 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon, δ 1.18 (3H, s, Me-19), 1.62 (3H, s, Me-18), 1.90 (3H, s, COMe), 2.21 (3H, s, Me-21), 3.50 (brm H-3), 4.68 (1H, dd, J = 11.0 and 4.0 Hz, H-12), 5.34 (1H, m, H-6); ^{13}C NMR data of the aglycon, Table 1; ^{1}H and ^{13}C NMR data of the sugar moiety, Table

3; ESIMS *m/z* 1037 $[M + Na]^+$, 977 $[M - 60 + Na]^+$, 917 $[M - 60 - 60 + Na]^+$, 817 $[M - 60 - 160 + Na]^+$, 673 $[M - 60 - 160 - 144 + Na]^+$, 615 $[M - 422 + Na]^+$, 471 $[M - 422 - 144 + Na]^+$, 327 $[M - 422 - 144 - 144 + Na]^+$; *anal*. C 60.33%, H 8.15%, O 31.53%, calcd for $C_{51}H_{82}O_{20}$ C 60.34%, H 8.14%, O 31.52%.

Compound 12: amorphous powder; $[\alpha]_D^{25}$ +99 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **9**; ^1H and ^{13}C NMR data of the sugar moiety, Table 4; ESIMS *m/z* 1205 $[M + Na]^+$, 1083 $[M - 122 + Na]^+$, 961 $[M - 122 - 122 + Na]^+$, 801 $[M - 122 - 122 - 160 + Na]^+$, 657 $[M - 122 - 122 - 160 - 144 + Na]^+$, 615 $[M - 590 + Na]^+$, 471 $[M - 590 - 144 + Na]^+$, 327 $[M - 590 - 144 - 144 + Na]^+$; *anal*. C 63.92%, H 7.68%, O 28.41%, calcd for $C_{63}H_{90}O_{21}$ C 63.94%, H 7.67%, O 28.39%.

Compound 13: amorphous powder; $[\alpha]_D^{25}$ +133 (*c* 0.1, MeOH); ^1H NMR data of the aglycon, δ 1.18 (3H, s, Me-19), 1.66 (3H, s, Me-18), 2.23 (3H, s, Me-21), 3.49 (brm H-3), 4.72 (1H, dd, $J = 11.0$ and 4.0 Hz, H-12), 5.37 (1H, m, H-6), 6.40 (1H, d, $J = 16.0$ Hz, H-2^I), 7.68 (1H, d, $J = 16.0$ Hz, H-3^I), 6.90 (2H, d, $J = 8.0$ Hz, H-5^I, H-9^I), 7.50 (2H, d, $J = 8.0$ Hz, H-6^I, H-8^I); ^{13}C NMR data of the *p*-coumaric moiety, δ 169.0 (C-1^I), 116.0 (C-2^I), 145.9 (C-3^I), 125.3 (C-4^I), 130.5 (C-5^I, C-9^I), 116.5 (C-6^I, C-8^I), 159.0 (C-4^I); ^1H and ^{13}C NMR data of the sugar moiety, Table 4; ESIMS *m/z* 1143 $[M + Na]^+$, *anal*. C 61.04%, H 7.57%, O 31.41%, calcd for $C_{57}H_{84}O_{22}$ C 61.06%, H 7.55%, O 31.39%.

Compound 14: amorphous powder; $[\alpha]_D^{25}$ +77 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **3**; ^1H and ^{13}C NMR data of the sugar moiety, Table 5; ESIMS *m/z* 1451 $[M + Na]^+$, 1303 $[M - 148 + Na]^+$, 1259 $[M - 148 - 44 + Na]^+$, 1097 $[M - 148 - 44 - 162 + Na]^+$, 935 $[M - 148 - 44 - 162 - 162 + Na]^+$, 939 $[M - 512 + Na]^+$, 795 $[M - 512 - 144 + Na]^+$, 651 $[M - 512 - 144 - 144 + Na]^+$, 491 $[M - 512 - 144 - 144 - 160 + Na]^+$; *anal*. C 58.80%, H 7.63%, O 33.58%, calcd for $C_{70}H_{108}O_{30}$ C 58.81%, H 7.61%, O 33.57%.

Compound 15: amorphous powder; $[\alpha]_D^{25}$ +89 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **3**; ^1H and ^{13}C NMR data of the sugar moiety, Table 5; ESIMS *m/z* 1435 $[M + Na]^+$, 1287 $[M - 148 + Na]^+$, 1243 $[M - 148 - 44 + Na]^+$, 1081 $[M - 148 - 44 - 162 + Na]^+$, 919 $[M - 148 - 44 - 162 - 162 + Na]^+$, 923 $[M - 512 + Na]^+$, 779 $[M - 512 - 144 + Na]^+$, 635 $[M - 512 - 144 - 144 + Na]^+$, 491 $[M - 512 - 144 - 144 - 144 + Na]^+$; *anal*. C 59.45%, H 7.72%, O 32.84%, calcd for $C_{70}H_{108}O_{29}$ C 59.48%, H 7.70%, O 32.82%.

Compound 16: amorphous powder; $[\alpha]_D^{25}$ +107 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **2**; ^1H and ^{13}C NMR data of the sugar moiety, Table 5; ESIMS *m/z* 1433 $[M + Na]^+$, 1285 $[M - 148 + Na]^+$, 1225 $[M - 148 - 60 + Na]^+$, 1123 $[M - 148 - 162 + Na]^+$, 961 $[M - 148 - 162 - 162 + Na]^+$, 923 $[M - 510 + Na]^+$, 779 $[M - 510 - 144 + Na]^+$, 635 $[M - 510 - 144 - 144 + Na]^+$, 491 $[M - 510 - 144 - 144 - 144 + Na]^+$; *anal*. C 59.55%, H 7.59%, O 32.89%, calcd for $C_{70}H_{106}O_{29}$ C 59.56%, H 7.57%, O 32.87%.

Compound 17: amorphous powder; $[\alpha]_D^{25}$ +113 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **2**; ^1H and ^{13}C NMR data of the sugar moiety, Table 5; ESIMS *m/z* 1449 $[M + Na]^+$, 1301 $[M - 148 + Na]^+$, 1241 $[M - 148 - 60 + Na]^+$, 1139 $[M - 148 - 162 + Na]^+$, 977 $[M - 148 - 162 - 162 + Na]^+$, 939 $[M - 510 + Na]^+$, 795 $[M - 510 - 144 + Na]^+$, 651 $[M - 510 - 144 - 144 + Na]^+$, 491 $[M - 510 - 144 - 144 - 160 + Na]^+$; *anal*. C 58.87%, H 7.49%, O 32.65%, calcd for $C_{70}H_{106}O_{30}$ C 58.89%, H 7.48%, O 33.62%.

Compound 18: amorphous powder; $[\alpha]_D^{25}$ +169 (*c* 0.1, MeOH); NMR data of the aglycon moiety identical to those of compound **11**; ^1H and ^{13}C NMR data of the sugar moiety, Table 4; ESIMS *m/z* 1023 $[M + Na]^+$, 963 $[M - 60 + Na]^+$, 903 $[M - 60 - 60 + Na]^+$; *anal*. C 59.96%, H 8.08%, O 31.97%, calcd for $C_{50}H_{80}O_{20}$ C 59.98%, H 8.05%, O 31.96%.

Acid Hydrolysis of 1−18. Each compound (6 mg) was heated at 60 °C for 6 h with dioxane (2 mL) and 0.2 N H_2SO_4 (1 mL) to yield the aglycon and sugar units. After hydrolysis, the reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was neutralized and the eluate concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$−MeOH−H_2O (7:1:1.2, bottom layer) system to obtain sugars. Cymarose, oleandrose, thevetose, canarose, and digitalose were assigned to the D series on the basis of their optical rotation values:[7] D-cymarose, $[\alpha]_D^{25}$ +54.4 (*c* 0.1, H_2O); D-oleandrose, $[\alpha]_D^{25}$ −12.5 (*c* 0.1, H_2O); D-digitalose, $[\alpha]_D^{25}$ +75.4 (*c* 0.1, H_2O); D-thevetose, $[\alpha]_D^{25}$ +43.4 (*c* 0.1, H_2O); D-canarose, $[\alpha]_D^{25}$ −85.0 (*c* 0.1, H_2O).

GC Analysis for Determination of Glucose Absolute Configuration. A solution of each compound (2 mg) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evapored under a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a 1-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolyzate were detected by comparison with the retention time of an authentic sample of D-glucose (Sigma Aldrich, Milan, Italy) after treatment with 1-(trimethylsilyl)imidazole in pyridine.[8]

Antiproliferative Activity Assay. J774.A1 (murine monocyte/macrophage), WEHI-164 (murine fibrosarcoma), and HEK-293 (human epithelial kidney) cells were grown as reported previously.[9] All reagents for cell culture were from Hy-Clone (Euroclone, Paignton, Devon, UK); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] and 6-mercaptopurine (6-MP) were from Sigma Chemicals (Milan, Italy). J774.Al, WEHI-164, and HEK-293 (3.4×10^4 cells) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in 5% CO_2 and 95% air for 2 h. Thereafter, the medium was replaced with 50 μL of fresh medium and a 75 μL aliquot of 1:4 serial dilution of each test compound was added, and then the cells were incubated for 72 h. In some experiments, serial dilutions of 6-MP were added. The cell viability was assessed through an MTT conversion assay.[9−11] The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-MP was calculated as follows: % dead cells = 100 − (OD treated/OD control) × 100. Table 6 shows the results obtained expressed as an IC_{50} value (μM), the concentration that inhibited cell growth by 50% as compared to the control.

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