Full Papers

New Polyoxypregnane Ester Derivatives from *Leptadenia hastata*

Rita Aquino*

Dipartimento di Chimica delle Sostanze Naturali, Università Federico II, via D. Montesano 49, 80131 Napoli, Italy

Gianfranco Peluso

Istituto Biochimica delle Proteine ed Enzimologia, Via Troiano 6, Arco Felice 80072 Napoli, Italy

Nunziatina De Tommasi, Francesco De Simone, and Cosimo Pizza

Centro Interdipartimentale di Chimica, Biologia e Tecnologia Farmaceutiche, Università degli Studi di Salerno, P.zza V. Emanuele 9, 84084, Penta di Fisciano, Salerno, Italy

Received August 10, 1995[⊗]

Following the characterization of polyoxypregnane esters and esterified glycosides from the less polar extracts of *Leptadenia hastata*, 34 new congeners have been isolated from the more polar extracts. They possess sarcostin or deacetylmetaplexigenin as the aglycons and acetyl, benzoyl, cinnamoyl, nicotinoyl, and *m*-hydroxybenzoyl residues as the ester moieties linked at C-12 and/or C-20 of the aglycons. The oligosaccharide moiety linked to C-3 of the aglycons was made up of three to five 2,6-dideoxy-3-*O*-methylpyranoses, 6-deoxy-3-*O*-methylpyranoses, or glucose. Their structures have been elucidated on the basis of spectral evidence. Glycosides with more complex oligosaccharidic chains were tested for their antitumor activity on Raji cells.

The plants of the family Asclepiadaceae are known to contain cytotoxic and tumoricidal *C*/*D*-*cis*-polyoxypregnane esters and glycosides.[1−4] During the course of our investigation of new potentially bioactive principles from *Leptadenia hastata* Decne. (Asclepiadaceae), we reported the isolation and structural elucidation of six new *C*/*D*-*cis*-polyoxypregnane ester derivatives **1**−**6** together with five known esters **10**−**14** from the less polar fractions of the chloroform extract of *L. hastata*.[1] Three new glycosides **7**−**9** which linked one or two sugar units such as D-cymarose and D-oleandrose at C-3 of the aglycon were also present in the more polar fraction of the same extract.[1]

Further investigations of the most polar extracts ($CHCl_3$/MeOH 9:1 and MeOH) have led to the isolation of 34 new related esterified aglycons and glycosides (compounds **15**−**48**, Figure 1). The structures of these compounds are based on the known polyoxypregnane skeleton of sarcostin or deacetylmetaplexigenin as well as acetyl, benzoyl, cinnamoyl, nicotinoyl, and *m*-hydroxybenzoyl ester moieties linked at C-12 and/or at C-20 of the aglycons. In addition, the glycosides **18**−**48** which possess an oligosaccharide portion linked at C-3 to the aglycons consist of three to five deoxyhexopyranoses and hexopyranoses, sugars well known to occur in Asclepiadaceae plants.

This paper deals with the isolation and structural elucidation of these esters and glycosides and with evaluation of antitumor activity on Raji cells (human lymphoblastoid cell line from Burkitt lymphoma) on the glycosides with more complex oligosaccharide chains.

* To whom correspondence should be addressed. Phone: 39 81 7486542. Fax: 39 81 7486510.

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1996.

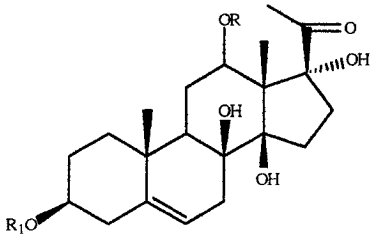
Results and Discussion

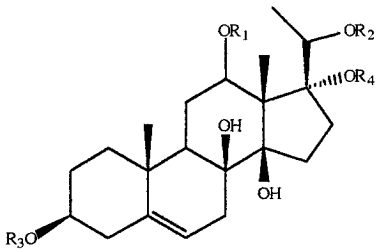
A Sephadex LH-20 column, repeated droplet counter current chromatographic (DCCC) separations, and a final purification by reversed-phase HPLC of the $CHCl_3$/MeOH (9:1) and MeOH extracts of *L. hastata* provided three new *C*/*D*-*cis*-polyoxypregnane esters **15**−**17** and 31 new esterified glycosides **18**−**48**.

Compounds 15−**17.** MS and NMR data of compounds **15**−**17** indicated that they were unidentified derivatives of the *C*/*D*-*cis*-polyoxypregnane sarcostin (3β,8β,12β,14β,17β,20-hexahydroxypregn-5-ene) by comparison with data previously reported for compounds **1**−**5**[1] and literature data.[2,3] In addition to the pregnene moiety, the ^{1}H and ^{13}C NMR spectra (Table 1 and Experimental Section) of **15** showed signals due to cinnamoyl and benzoyl groups; the spectra of **16** indicated the presence of a nicotinoyl and a cinnamoyl group, and the spectra of **17** ($C_{25}H_{38}O_8$ from EIMS and ^{13}C and DEPT ^{13}C NMR) suggested two acetyl residues. The ester linkages were located at C-12 and C-20 on the basis of the chemical shifts of the double doublet (δ 4.75, 4.45, and 4.73, respectively, for compounds **15**−**17**, J = 12 and 4 Hz) and the quartet (δ 5.03, 4.85, and 4.65, respectively, J = 6.5 Hz) signals ascribable to esterified H-12 and H-20 of sarcostin. These observations led to the conclusion that the structure of compound **17** was 12,20-di-*O*-acetylsarcostin.

The spectra of compound **15** ($C_{37}H_{44}O_8$ from EIMS and ^{13}C and DEPT ^{13}C NMR) with respect to compound **3** (12-*O*-benzoyl-20-*O*-cinnamoylsarcostin)[1] revealed some diagnostic differences in the resonances of H-20 of sarcostin and H-2″ and H-3″ of the cinnamoyl residue. The signal assigned to the H-20 proton (δ 5.03) was shifted downfield by 0.18 ppm in compound **15** relative to the same hydrogen (δ 4.85) in compound **3**. As

S0163-3864(96)00251-0 CCC: $12.00





Compounds	R	R1
6	Nic	H
12	Cinn	H
13	Ac	H
14	Bz	H
29	Cinn	I
30	Ac	I
32	Ac	C
33	Bz	C
37	Bz	G
38	Nic	G
44	Bz	W
45	Ac	W
46	Cinn	W

Compounds	R1	R2	R3	R4
1	Ac	H	H	H
2	Bz	Bz	H	H
3	Bz	Cinn	H	H
4	Cinn	Ac	H	H
5	Nic	Ac	H	H
7	Bz	Cinn	A	H
8	Cinn	H	B	H
9	Bz	Cinn	B	H
10	Cinn	H	H	H
11	Cinn	Nic	H	H
15	Cinn	Bz	H	H
16	Nic	Cinn	H	H
17	Ac	Ac	H	H
18	Cinn	H	D	H
19	Bz	Cinn	D	H
20	Cinn	H	E	H
21	Bz	Cinn	E	H
22	Bz	Bz	E	H

Compounds	R1	R2	R3	R4
23	Cinn	Ac	E	H
24	Cinn	H	I	H
25	Bz	Cinn	I	H
26	Ac	H	I	H
27	Nic	Ac	I	H
28	Bz	H	I	H
31	Bz	Cinn	L	H
34	Bz	Cinn	G	H
35	Bz	Bz	G	H
36	Cinn	Nic	G	H
39	Cinn	m-OH-Bz	G	H
40	Ac	H	W	H
41	Cinn	H	W	H
42	Nic	Ac	W	H
43	Cinn	Bz	W	H
47	Nic	Cinn	W	Ac
48	Cinn	H	F	H

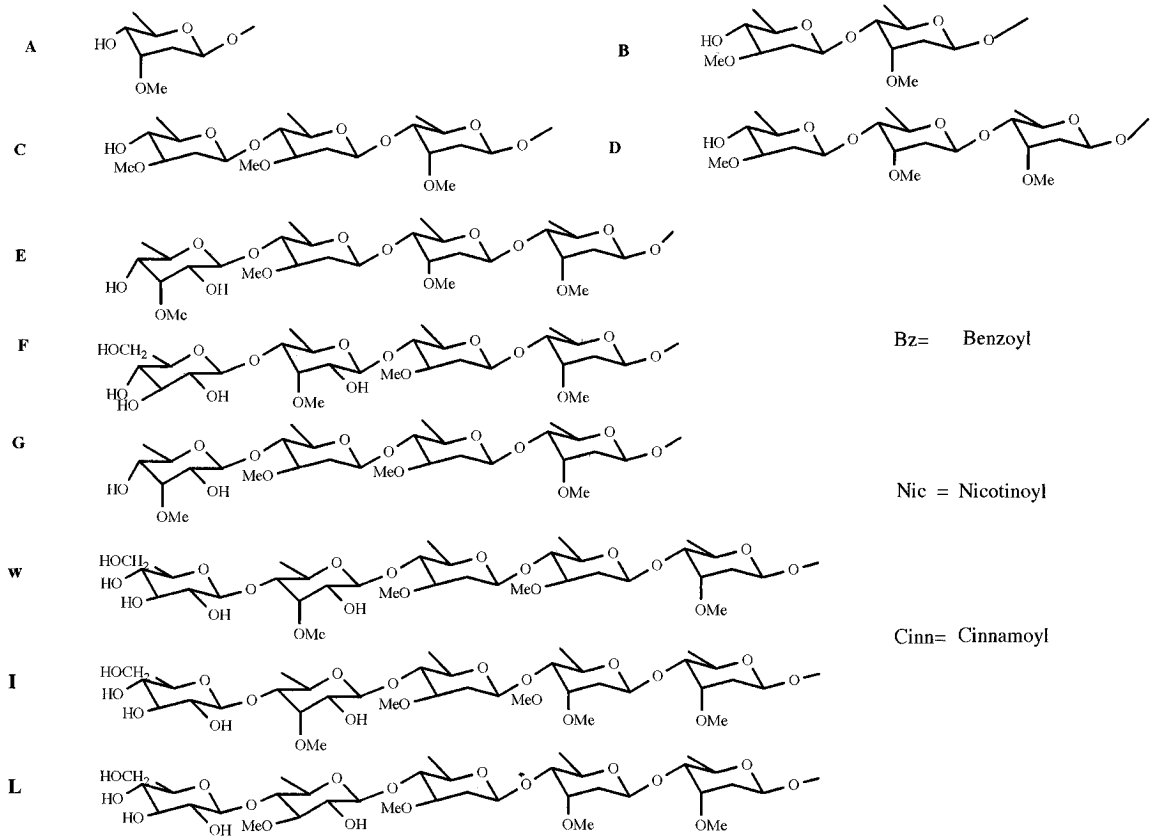


Figure 1.

previously noted[1] this remarkable downfield shift of the proton at the esterified C-20 position can be explained by the presence of a benzoyl group at C-20. Moreover, the resonances of H-2″ and H-3″ at δ 6.67 and 6.81, respectively, which are almost superimposable on those observed in 12-*O*-cinnamoyl derivatives such as compounds **8**, **10**, and **11** indicated a cinnamoyl residue esterified at C-12, whereas in the 20-*O*-cinnamoyl

Table 1. ^{13}C NMR Data for Aglycon Moieties of Compounds **15**−**17**, **28**, **39**, **43**, and **47** (500 MHz in CD_3OD)

position		**15**	**16**	**17**	**28**	**39**	**43**	**47**
1		39.60	39.80	39.80	38.60	38.70	38.70	38.60
2		31.50	31.70	31.70	30.60	31.00	31.00	30.60
3		72.30	72.40	72.50	79.20	79.20	79.30	79.20
4		42.90	42.90	42.90	37.90	38.00	38.00	38.00
5		140.00	130.00	140.10	139.90	140.00	140.00	139.90
6		119.50	118.80	119.30	119.70	119.70	119.70	119.70
7		34.30	33.50	34.30	34.30	34.30	34.30	33.50
8		75.30	75.00	75.00	75.00	75.30	75.30	75.00
9		44.70	45.20	44.70	44.60	44.60	44.70	45.20
10		36.80	36.86	36.70	38.00	36.90	36.90	36.86
11		26.10	25.00	25.00	25.91	26.00	26.00	25.00
12		76.30	73.60	73.60	76.20	76.30	76.40	73.60
13		57.10	56.20	56.10	57.20	57.10	57.10	56.30
14		88.60	88.50	88.60	88.40	88.50	88.60	88.60
15		35.20	35.20	35.50	35.10	35.30	35.30	35.20
16		34.10	34.10	34.10	34.20	34.20	34.10	33.10
17		89.95	89.65	89.49	89.90	89.90	89.90	91.00
18		11.40	11.30	11.30	11.30	11.40	11.30	11.30
19		18.60	18.30	18.30	18.30	18.60	18.30	18.60
20		74.20	75.30	75.00	71.80	74.20	74.20	74.10
21		15.20	15.20	15.00	18.70	15.20	15.30	15.30
CH_3CO				178.20[b]				178.00[b]
CH_3CO				21.80[b]				21.70[b]
Cinn	1	167.10	167.20			167.20	167.00	167.30
	2	118.00	118.50			118.40	119.00	118.90
	3	146.10	147.00			146.30	146.20	146.30
	4	135.40	135.00			135.40	135.00	136.00
	5	129.60[b]	129.10[b]			129.70[b]	129.50[b]	129.90[b]
	6	128.80[b]	128.80[b]			128.90[b]	128.90[b]	128.70[b]
	7	131.70	131.70			131.00	131.10	131.70
	8	128.80[b]	128.80[b]			128.90[b]	128.90[b]	128.70[b]
	9	129.60[b]	129.10[b]			129.70[b]	129.50[b]	129.90[b]
Bz	1	168.20			168.50		168.10	
	2	130.90			131.10		131.00	
	3	129.80[b]			129.90[b]		129.70[b]	
	4	130.10[b]			130.20[b]		130.20[b]	
	5	134.20			134.60		134.10	
	6	130.10[b]			130.20[b]		130.30[b]	
	7	129.80[b]			129.90[b]		129.70[b]	
Nic	2		153.70[b]					153.80[b]
	3		127.20					127.30
	4		136.50					136.30
	5		123.70					123.70
	6		153.60[b]					153.80[b]
	7		164.80					164.70
m-OHBz								
	1					168.00		
	2					116.00		
	3					134.10		
	4					153.20		
	5					121.20		
	6					131.00[b]		
	7					125.90[b]		

[a] Assignments confirmed by ^{13}C DEPT, HETCOR, and COLOC experiments. [b] Double signals.

derivatives **3**, **7** and **9**, H-2″ and H-3″ were observed to resonate at 6.12 and 6.50 ppm. The relative positions of the cinnamoyl and benzoyl residues were confirmed by the results of a COLOC (2D $^1H-^{13}C$ shift correlation spectrum by long-range couplings) experiment which showed clear long-range correlation peaks between the carbonyl carbon of cinnamoyl (167.0 ppm) and both H-2″ (6.67 ppm, d, $J = 15.9$ Hz) of the cinnamoyl residue and H-12 (4.75 ppm) of the aglycon. Therefore, compound **15** is 12-*O*-cinnamoyl-20-*O*-benzoylsarcostin.

Compound **16** has the same molecular formula ($C_{36}H_{43}O_8N$ from EIMS and NMR analysis) and ester residues as those of compound **11** (gagaminin) based on the almost superimposable MS and NMR data.[1,5] Nevertheless, COLOC experiments indicated that compound **16** is 12-*O*-nicotinoyl-20-*O*-cinnamoylsarcostin (isogagaminin) as it showed a long-range correlation between the carbonyl group of cinnamic acid (167.0 ppm) and both H-2″ (δ 6.12) of the cinnamoyl and H-20 (δ 4.85) of the aglycon.

Compounds 18−19 (Chain D). Compounds **18** and **19** showed quasimolecular $[M - H]^-$ ion peaks at *m/z* 943 and 1047 by FABMS in the negative ion mode and gave ^{13}C and DEPT ^{13}C NMR data consistent with $C_{51}H_{76}O_{16}$ and $C_{58}H_{80}O_{17}$ molecular formulas, respectively. An examination of their NMR spectra revealed signals due to aglycon protons and carbons identical with those observed in 12-*O*-cinnamoylsarcostin (penupogenin) (**10**) and 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (**3**) isolated from the same plant.[1] In their ^{13}C NMR spectra glycosylation shifts of C-2 (*ca.* −1.2 ppm), C-3 (*ca.* +8.0 ppm), and C-4 (*ca.* −4.6 ppm) relative to the respective aglycons revealed that they are glycosylated at position 3, a general feature of the pregnane glycosides. Their FABMS spectra also showed a similar fragmentation pattern arising from the successive losses of one (144), two (2 × 144), and three (3 × 144) mass units corresponding to successive loss of 3-*O*-methyl-2,6-dideoxyhexopyranose units from the quasimolecular ions. In addition to the aglycon signals, ^{13}C NMR spectra of both **18** and **19** exhibited 21 signals ascribable to the saccharide portion made up of three 3-*O*-methyl-2,6-dideoxyhexopyranose units.[6] Three signals were assigned to anomeric carbons (97.10, 99.40, and 102.65 ppm), three to methoxyl (57.26, 58.30, and 58.42 ppm), and three to methyl signals (18.30, 18.60, and 18.21 ppm). In their ^1H NMR spectra three methoxyl (δ 3.45, 3.46, and 3.48), three secondary methyl (δ 1.18, 1.16, and 1.28), and three anomeric proton signal (δ 4.90, 4.82 and 4.55, each dd, $J \approx 9.5$ and 2.0 Hz), typical of 2,6-dideoxyhexopyranoses, also supported the above results. All proton signals due to the three sugar units (Tables 2 and 3) were assigned by a COSY ($^1H-^1H$ direct chemical shift correlation spectroscopy) experiment which allowed the sequential assignments of hydrogens from H-1 to H-6 within each sugar unit starting from the well-separated anomeric signals. The β-linkages of the three sugar units were shown by the large ($J \approx 9.5$ 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.[7,8] A HETCOR ($^1H-^{13}C$ direct chemical shift correlation spectroscopy) correlated each hydrogen signal to the corresponding carbon signal and allowed the assignment of all the resonances and therefore to the identification of the sugars as a terminal β-D-oleandropyranosyl and two inner β-D-cymaropyranosyl units each glycosidated at C-4. Moreover, because D-cymarose possess only two hydroxyl groups at C-1 and C-4, the sugar sequence has to be linear.

It is interesting to note that C-1 of D-cymarose characteristically resonates upfield (97.10 ppm) when linked at C-3 of the aglycon in contrast with the resonance at *ca.* 99.4 ppm when it is linked to the hydroxyl group of a different sugar.[9] On the basis of this NMR evidence, compound **18** was determined to be penupogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and compound **19** to be 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin 3-*O*-β-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compounds 20−23 (Chain E). Compounds **20**−**23**

Table 2. ^{13}C NMR Data of Sugar Moieties of Compounds **18**−**48**[a] (500 MHz in CD_3OD)

position		**18**−**19** chain D δ C	**20**−**23** chain E δ C	**31** chain L δ C	**24**−**30** chain I δ C		**32**−**33** chain C δ C	**34**−**39** chain G δ C	**40**−**47** chain W δ C	**48** chain F δ C
D-Cym	1	97.10	97.20	97.03	97.20	D-Cym 1	97.27	97.21	97.28	97.16
	2	37.80	36.62	36.41	36.62	2	36.74	36.74	36.82	36.10
	3	78.80	78.45	78.30	78.45	3	78.54	78.54	78.05	78.40
	4	83.00	83.82	83.83	83.82	4	83.94	83.94	83.66	83.10
	5	69.98	69.98	69.82	69.60	5	69.99	69.98	69.60	69.84
	6	18.30	18.50	18.45	18.50	6	18.49	18.49	18.70	18.09
−OMe		57.26	58.40	57.40	58.40	−OMe	57.77	57.77	58.55	57.61
D-Cym	1	99.40	101.16	101.16	101.16	D-Ole 1	102.70	102.70	102.10	102.57
	2	37.33	37.66	37.88	37.60	2	38.08	38.08	37.79	36.10
	3	78.80	78.56	78.89	78.56	3	80.55	80.55	80.55	80.10
	4	82.30	83.00	83.00	83.00	4	84.02	84.01	83.66	83.22
	5	69.40	69.95	70.00	69.95	5	71.00	71.27	71.99	71.96
	6	18.60	18.60	18.60	18.60	6	18.50	18.52	18.74	18.09
−OMe		58.30	58.30	57.20	58.30	−OMe	57.70	57.74	57.64	58.62
D-Ole	1	102.65	101.90	102.50	101.90	D-Ole 1	102.68	102.29	102.63	
	2	36.30	36.41	36.41	36.41	2	37.52	39.96	39.82	
	3	81.49	80.36	80.36	80.38	3	81.59	80.14	80.10	
	4	76.85	83.55	83.73	83.55	4	76.80	84.09	83.66	
	5	73.13	71.25	71.71	71.25	5	73.05	72.35	71.20	
	6	18.28	18.74	18.70	18.74	6	18.30	18.79	18.00	
−OMe		58.42	57.40	57.40	57.50	−OMe	58.41	57.52	58.55	
D-Allo	1		102.69		102.66	D-Allo 1		102.27	102.10	102.10
	2		73.66		72.64	2		72.61	73.05	72.20
	3		83.00		83.15	3		83.00	84.00	83.22
	4		75.25		84.35	4		75.31	83.26	83.81
	5		72.25		71.99	5		73.65	72.38	71.96
	6		18.94		18.30	6		18.92	18.83	18.09
−OMe			62.47		61.96	−OMe		62.55	61.96	61.93
D-The	1			101.09						
	2			72.79						
	3			87.17						
	4			83.00						
	5			71.40						
	6			17.82						
−OMe				61.84						
D-Glu	1			106.08	106.65	D-Glu 1			106.25	106.19
	2			75.36	75.18	2			75.61	75.02
	3			77.89	78.00	3			78.05	78.02
	4			72.50	73.00	4			72.75	72.20
	5			77.89	78.40	5			78.58	78.49
	6			62.92	63.10	6			63.17	63.11

[a] Assignments confirmed by DEPT and HETCOR experiments.

have molecular formula $C_{58}H_{88}O_{20}$, $C_{65}H_{92}O_{21}$, $C_{63}H_{90}O_{21}$, and $C_{60}H_{90}O_{21}$, respectively, deduced from MS and NMR analysis. Their aglycons were identified as penupogenin (**10**), 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (**3**), 12,20-di-*O*-benzoylsarcostin (**2**), and 12-*O*-cinnamoyl-20-*O*-acetylsarcostin (**4**), respectively, by NMR data.[1] A detailed comparison of their NMR spectra showed that the sugar chain was identical in the four compounds. Their FABMS spectra displayed prominent fragment peaks ascribable to the successive lossess of 160, 144, 2 × 144, and 3 × 144 mass units from the quasimolecular ions. The former peak can be attributed to the cleavage of a terminal 3-methoxy-4-deoxyhexopyranose and the latter three peaks to the subsequent losses of three 3-methoxy-2,6-dideoxyhexopyranosyl units. ^1H and ^{13}C NMR confirmed that **20**−**23** are tetrasaccharides made up of three 3-*O*-methyl-2,6-dideoxyhexopyranosyl and a 3-*O*-methyl-6-deoxyhexopyranosyl units by the presence of an anomeric carbon (^1H NMR δ 4.75, 1H, d, J = 7.0 Hz; ^{13}C 102.69 ppm), a doublet Me (1.32, 3 H, δ, J = 6.0 Hz), and a *O*-methoxyl (^1H NMR δ 3.62, 3 H, s; ^{13}C 62.47 ppm) with the additional signals observed in the sugar chain which are also seen in compounds **18** and **19**. The structure of the tetrasaccharide chain was achieved by 2D NMR spectral data including $^1H-^1H$ COSY, $^1H-^{13}C$ HETCOR, and ROESY (rotating-frame Overhauser enhancement spectroscopy) from the most abundant glycoside **20**.

Evaluation of the COSY spectrum of **20** led to the sequential assignment of the resonances of each sugar hydrogen signal starting from H-1. Observation of the clear cross peaks between directly correlated hydrogens and carbons in the HETCOR spectrum led to the identification of the sugars as a terminal 6-deoxy-3-*O*-methyl-β-D-allopyranoside[10] as well as a β-D-oleandropyranosyl and two β-D-cymaropyranosyl inner units. The configurations of the sugars were deduced by the coupling constants of the anomeric protons and by the chemical shifts of the key carbons at position 2, 3, and 5. Each sugar unit was glycosidated at C-4 as shown by the glycosilation shifts observed for C-4′, C-4″, and C-4‴ (Table 2).

Direct evidence for the sugar sequence and their linkage sites was derived from the results of a ROESY experiment which showed correlation peaks between anomeric protons and protons linked to glycosylated carbons, for example, between H-1 (δ 4.75) of 6-deoxy-3-*O*-methylallose and H-4 (δ 3.18) of the oleandrose and between H-1 (δ 4.95) of the first unit of cymarose and H-3 (δ 3.15) of the aglycon. Thus, the sugar attached to the C-3 position of the aglycon was a cymarose unit with the anomeric carbon resonating at 97.7 ppm. The

Table 3. ^{1}H NMR Data of Sugar Moieties of Compounds **18**−**48**[a] (J_{HH} in Hz) (500 MHz in CD_3OD)

position		**18**−**19** chain D δH	**20**−**23** chain E δH	**31** chain L δH	**24**−**30** chain I δH	position		**32**−**33** chain C δH	**34**−**39** chain G δH	**40**−**47** chain W δH	**48** chain F δH
D-Cym	1	4.90 dd, 9.6, 2.0	4.95 dd, 9.6, 2.0	4.95 dd, 9.6, 2.0	4.95	D-Cym	1	4.89 dd, 9.6, 2.0	4.88 dd, 9.6, 2.0	4.90	4.89
	2	2.10;[b] 1.55[b]	2.05;[b] 1.48[b]	2.10;[b] 1.55[b]	2.05;[b] 1.48[b]		2	2.12;[b] 1.58[b]	2.10;[b] 1.52[b]	2.10;[b] 1.58[b]	2.10;[b] 1.55[b]
	3	3.88 q, 3.0	3.80 q, 3.0	3.80 q, 3.0	3.80		3	3.86 q, 3.0	3.82 q, 3.0	3.88	3.88
	4	3.30 dd, 9.5, 3.0	3.30 dd, 9.5, 3.0	3.30 dd, 9.5, 3.0	3.30		4	3.32 dd, 9.5, 3.0	3.31 dd, 9.5, 3.0	3.32	3.32
	5	3.90 dq, 9.5, 6.4	3.90 dq, 9.5, 6.4	3.90 dq, 9.5, 6.4	3.90		5	3.90 dq, 9.5, 6.4	3.88 dq, 9.5, 6.4	3.90	3.92
	6	1.18 d, 6.4	1.18 d, 6.4	1.18 d, 6.4	1.17		6	1.18 d, 6.4	1.18 d, 6.4	1.18	1.18
−OMe		3.42 s	3.46 s	3.46 s	3.46	−OMe		3.42 s	3.46 s	3.42	3.42
D-Cym	1	4.82 dd, 9.0, 2.0	4.82 dd, 9.6, 2.0	4.82 dd, 9.1, 1.5	4.83	D-Ole	1	4.60 dd, 9.1, 1.5	4.61 dd, 9.1, 1.5	4.64	4.64
	2	2.15;[b] 1.62[b]	2.13;[b] 1.60[b]	2.05;[b] 1.48[b]	2.13;[b] 1.60[b]		2	2.42;[b] 1.52[b]	2.41;[b] 1.50[b]	2.45;[b] 1.54[b]	2.45;[b] 1.54[b]
	3	3.86 q, 3.0	3.86 q, 3.0	3.86 q, 3.0	3.86		3	3.38[b]	3.38[b]	3.31[b]	3.30[b]
	4	3.34 dd, 9.5, 3.0	3.40 dd, 9.5, 3.0	3.40 dd, 9.5, 3.0	3.40		4	3.22 t, 9.5	3.18 t, 9.5	3.19	3.18
	5	3.80 dq, 9.5, 6.4	3.82 dq, 9.5, 6.4	3.80 dq, 9.5, 6.4	3.82		5	3.80 dq, 9.5, 6.5	3.80 dq, 9.5, 6.5	3.80	3.80
	6	1.16 d, 6.4	1.17 d, 6.4	1.18 d, 6.4	1.17		6	1.28 d, 6.5	1.28 d, 6.5	1.28	1.28
−OMe		3.45 s	3.46 s	3.46 s	3.45 s	−OMe		3.46 s	3.44 s	3.46	3.46
D-Ole	1	4.55 dd, 9.1, 1.5	4.61 dd, 9.1, 1.5	4.61 dd, 9.1, 1.5	4.61	D-Ole	1	4.55 dd, 9.1, 2.5	4.64 dd, 9.1, 1.5	4.61	
	2	2.35;[b] 1.45[b]	2.40;[b] 1.52[b]	2.40;[b] 1.52[b]	2.40;[b] 1.52[b]		2	2.33;[b] 1.43[b]	2.45;[b] 1.54[b]	2.42;[b] 1.52[b]	
	3	3.30[b]	3.38[b]	3.38[b]	3.38[b]		3	3.30[b]	3.30[b]	3.38[b]	
	4	3.18 t, 9.5	3.18 t, 9.5	3.18 t, 9.5	3.16		4	3.18 t, 9.5	3.18 t, 9.5	3.18	
	5	3.40 dq, 9.5, 6.2	3.80 dq, 9.5, 6.5	3.80 dq, 9.5, 6.2	3.82		5	3.40 dq, 9.5, 6.2	3.80 dq, 9.5, 6.2	3.80	
	6	1.28 d, 6.2	1.28 d, 6.5	1.28 d, 6.2	1.28		6	1.28 d, 6.2	1.28 d, 6.2	1.28	
−OMe		3.47 s	3.44 s	3.44 s	3.44	−OMe		3.47 s	3.46 s	3.44	
D-Allo	1		4.75 d, 7.0		4.75	D-Allo	1		4.74 d, 7.0	4.75	4.74
	2		3.22 dd, 7.0, 2.5		3.22		2		3.22 dd, 7.0, 2.5	3.24	3.22
	3		3.98 dd, 3.5, 2.5		3.98		3		3.98 dd, 3.5, 2.5	3.98	3.98
	4		3.82 dd, 9.0, 3.5		3.85		4		3.84 dd, 9.0, 3.5	3.84	3.84
	5		3.38 m		3.39		5		3.38 m	3.38	3.38
	6		1.39 d, 6.0		1.39		6		1.40 d, 6.0	1.38	1.38
−OMe			3.62 s		3.62	−OMe			3.63 s	3.64	3.63
D-The	1			4.33 d, 7.0							
	2			3.20 dd, 10.0, 7.0							
	3			3.46 t, 10.0							
	4			3.81 t, 10.0							
	5			3.39 m							
	6			1.15 d, 6.0							
−OMe				3.65 s							
D-Glu	1			4.44 d, 7.5	4.42	D-Glu	1			4.42	4.44
	2			3.43 dd, 7.5, 9.5	3.45		2			3.43	3.45
	3			3.48 d, 9.5	3.43		3			3.46	3.48
	4			3.31 d, 9.5	3.32		4			3.32	3.33
	5			3.26 m	3.28		5			3.27	3.26
	6			3.90 dd, 12.0, 5.0	3.91		6			3.88	3.94
				3.68 dd, 12.0, 3.5	3.64					3.60	3.72

[a] Assignments confirmed by COSY, HOHAHA. [b] Overlapped signals.

terminal sugar (6-deoxy-3-*O*-methylallose) was deduced to be attached to C-4 of the oleandrose unit which, in turn, links the second unit of cymarose. On the basis of the above evidence, the structure of the sugar chain of compounds **20**−**23** was determined to be 6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compounds 24−30 (Chain I). Compounds **24**−**30** possess the same pentasaccharide sugar chain attached to the C-3 position of the aglycons. They were identified from NMR data[1] as five sarcostin derivatives, penupogenin (**10**), 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (**3**), 12-*O*-acetylsarcostin (**1**), 12-*O*-nicotinoyl-20-*O*-acetylsarcostin (**5**), and the new aglycon 12-*O*-benzoylsarcostin and the two deacetylmetaplexigenin derivatives, kidjolanin (**12**) and metaplexigenin (**13**).

FABMS spectra of **24**−**30** showed a fragmentation pattern consistent with the cleavage of a terminal hexopyranosyl unit (162 mass units) followed by loss of a 3-*O*-methyl-6-deoxypyranose (160 mass units) and sequential loss of three 3-*O*-methyl-2,6-dideoxypyranoses (3 × 144 mass units).

^{1}H and ^{13}C NMR data suggested that **24**−**30** are pentasaccharide derivatives by the presence of five anomeric carbon and proton signals, one more than observed in compounds **20**−**23**. The additional sugar unit was identified as D-glucopyranose (Tables 2 and 3). Because the sugar region of the ^{1}H NMR spectra from δ 2.00 to 4.00 was complex and most of the shifts were overlapping, the proton coupling network was traced out by a combination of ^{1}H−^{1}H COSY, HOHAHA (2D homonuclear Hartmann Hahn spectroscopy), and ^{1}H−^{13}C HETCOR experiments on the major compound **24**. This led to the assignment of all the resonances and to the identification of the five sugar units. The HOHAHA spectrum, utilizing a mixing time of 100 ms, resolved the overlapped oligosaccharide region into subset of five monosaccharide spectra. Clear connectivities were observed from H-1 to H-6 of a D-glucose and a D-oleandrose unit whereas cross-peaks only from H-1 to H-3 were observed for two D-cymaroses and a 3-*O*-methyl-6-deoxy-D-allose. The coherence transfer to H-4 was not obtained because of the small $J_{H_3-H_4}$ in these last sugars. Once again, a COSY spectrum allowed the sequential assignments of most of the resonances within each sugar fragment; the proton resonances were associated with the corresponding carbons using HETCOR, and the results are provided in Tables 2 and 3. Data from the above experiments

led to the identification of two β-D-cymaropyranosyl, a β-D-oleandropyranosyl, and a 3-*O*-methyl-6-deoxy-β-D-allopyranosyl unit, all glycosidated at C-4. Thus, glucose was the terminal unit as suggested by the absence of any glycosidation shift, while, relative to compounds **20**−**23**, C-4 of allose was shifted downfield (β-effect) by 9.1 ppm whereas C-5 was shifted upfield (γ-effect) by 0.26 ppm as expected from a glycosylation shift. The β configurations at the anomeric carbons of the five sugars was determined by the *J* values of their anomeric proton signals (Table 2). The characteristic chemical shifts of the anomeric carbons and C-2, C-3, and C-5 of the deoxysugars together with C-3 and C-5 for glucose were also observed.[7,8] Finally. direct support for the attachment sites of each monosaccharide was obtained from the results of a ROESY spectrum which showed correlation peaks between the H-4 (δ 3.18) of D-oleandrose and H-1 (δ 4.75) of 3-*O*-methyl-6-deoxy-D-allose and between the H-1 (δ 4.61) of D-oleandrose and H-4 (δ 3.40) of the second unit of D-cymarose. Thus, the terminal glucose was linked to C-4 of the D-allose which was attached at C-4 of the D-oleandrose which, in turn, links the second unit of D-cymarose. Moreover, the chemical shift (97.50 ppm) of C-1′ of the first unit of D-cymarose confirmed that it was directly linked to the aglycon. These data led us to conclude that the structure of the sugar moiety of compounds **24**−**30** was β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compounds 32 and 33 (Chain C). Compounds **32** and **33** possess aglycons with an oxo group at C-20 identified as metaplexigenin (**13**) and cynanforidin (**14**) by NMR data.[1] They have trisaccharide sugar chains linked to C-3 of the aglycons as revealed by three anomeric signals (^{1}H NMR δ 4.89, 4.60 and 4.55, each dd, $J \approx 9.0$ and 2.0 Hz; ^{13}C δ 97.21, 102.70, and 102.68) in their spectra. All signals of the carbohydrate chain were identical in both compounds and corresponded with the saccharide arrangement (chain C) shown in Figure 1. The sugars were identified as β-D-cymaropyranosyl and two β-D-oleandropyranosyl units by comparison with literature data[6] and data reported for the sugar units of compounds **18** and **19**. D-Cymarose was directly linked to the aglycons (C-1′ 97.27 ppm), the second unit of D-oleandrose was terminal, and the first unit of D-oleandrose was glycosidated at C-4 as indicated by the downfield shift of C-4 (+7.22 ppm) and by the upfield shift (−1.04 and −1.05) experienced by C-3 and C-5 with respect to a terminal D-oleandrose (Table 3). Thus, **32** was metaplexigenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside and **33** was cynanforidin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound 31 (Chain L). The structure 12-*O*-benzoyl-20-*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 was assigned to glycoside **31** on the basis of the following evidence. Compounds **25** and **31** were shown to be isomers with identical molecular formula and fragmentation patterns in the FABMS spectrum which showed the sequential loss of a hexose (162 mass units), a 3-*O*-methyl-6-deoxypyranose (160 mass units), and three 3-*O*-methyl-2,6-dideoxypyranoses (3 × 144 mass units). The structural differences involve a different sugar in the tetrasaccharide moiety. The NMR spectra of **31** were very close to those of **25** except signals assigned to a 6-deoxy-3-*O*-methylallopyranose were missing in **31** and replaced by seven signals ascribable to a C-4 glycosylated thevetose on comparison with literature data.[11] To establish the nature of the sugar sequence, hypothesized by the fragmentation pattern in the FABMS spectrum, required analysis of the ROESY spectrum which showed correlation peaks between H-4 of oleandrose and H-1 of thevetose and between H-1 of oleandrose and H-4 of the second unit of cymarose.

Compounds 34−**39 (Chain G).** The aglycon moiety of compounds **34**−**38** was determined to be that of the known compounds 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (**3**), 12,20-*O*-dibenzoylsarcostin (**2**), 12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin (**11**), cynanforidin (**14**), and 12-*O*-nicotinoyl deacetylmetaplexigenin (**6**), respectively.[1] The aglycon moiety of compound **39** was a new sarcostin derivative. The 500 MHz NMR spectrum of **39** suggested the presence of a cinnamoyl and a *m*-hydroxybenzoyl (^{1}H NMR δ 6.80, br d, H-3″; 7.32, br m, H-5″; 7.50, t, J = 7.4 Hz, H-6″; 8.00, dd, J = 7.4, 1.2 Hz, H-7″) group. Also, ^{13}C NMR data (see Table 1) confirmed the presence of an *m*-hydroxybenzoyl[12] linked with an ester bond to sarcostin. By comparing NMR data of the sarcostin moiety of compound **39** with those of 12-*O*-cinnamoyl derivatives **8** and **15** and with those of 20-*O*-cinnamoyl derivatives such as **3**, **7**, and **19**,[1] the cinnamoyl residue was deduced to be at C-12 and the *m*-hydroxybenzoyl group linked to C-20.

NMR data also revealed that compounds **34**−**39** have the same tetrasaccharide sugar chain linked at C-3 of the aglycons. The presence of seven sugar carbon signals corresponding to those of a 3-*O*-methyl-6-deoxy-β-D-methylallopyranose[10] located this sugar at the terminal of the chain as found in compounds **20**−**23** (chain E), while the other sugar signals were assignable as two inner units of β-linked oleandropyranose and an inner unit of β-linked cymaropyranose. Accordingly, based on the resonance of C-1′ (97.21 ppm), D-cymarose was attached to C-3 of the aglycon. Two units of D-oleandrose were located at the center of the chain based on the observed glycosidation shifts at C-4, relative to the C-4 resonance of unglycosylated terminal oleandrose as shown in compounds **32** and **33** (Table 3). This structure was supported by the prominent fragment peaks due to loss of a terminal 3-*O*-methyl-6-deoxyhexopyranose (160 mass units) and to three 3-*O*-methyl-2,6-dideoxyhexopyranoses (3 × 144 mass units) in the FABMS spectrum. Thus, the structure of chain G was established as 6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Compounds 40−**47 (Chain W).** The NMR spectra revealed that the aglycon moieties of **40**−**46** were **1** (12-*O*-acetylsarcostin, **10** (penupogenin, **5** (12-*O*-nicotinoyl-20-*O*-acetylsarcostin), **15** (12-*O*-cinnamoyl-20-*O*-benzoylsarcostin), **14** (cyanforidin), **13** (metaplexigenin), and **12** (kidjolanin), respectively.[1] The genin of compound **47** presents an unusual acetylation at C-17 and was identified as 12-*O*-nicotinoyl-17-*O*-acetyl-20-*O*-cinnamoylsarcostin reported here for the first time (C-17

91.0 ppm, shifted *ca*. 1.0 ppm with respect to unacetylated model compounds).

^{1}H and ^{13}C NMR and FABMS spectra revealed that **40**−**47** are pentasaccharides having an identical sugar chain made up of D-cymarose, D-oleandrose, 3-*O*-methyl-6-deoxy-D-allose, and D-glucose. By means of COSY, HOHAHA, and HETCOR spectra recorded for the major compound **41**, each carbon and hydrogen signal was assigned as shown in Tables 2 and 3. Consequently it was possible to identify a terminal *β*-D-glucopyranose as well as an inner *β*-D-cymaropyranose, two *β*-D-oleandropyranoses, and a 3-*O*-methyl-6-deoxy-*β*-D-allopyranose, each glycosidated at C-4. NMR spectra also revealed that chain W is related to chain I by replacement of the second unit of cymarose with an oleandrose. Moreover, the sugar chain of compounds **40**−**47** was found to correspond to a 4‴-*O*-*β*-D-glucopyranosyl derivative of chain G. The sugar sequence was therefore established to be glucose−allose−oleandrose−oleandrose−cymarose by a ROESY experiment showing cross-peaks between H-4 of glucose and H-1 of 3-*O*-methyl-6-deoxy-*β*-D-allopyranose, between H-4 of the second unit of oleandrose and H-1 of 3-*O*-methyl-6-deoxy-*β*-D-allopyranose, and between H-1 of the first unit of oleandrose and H-4 of cymarose. Finally, confirmation of the sugar sequence was derived from the chemical shift of C-1′ in 3-*O*-methyl-6-deoxyallose (102.10 ppm). Previous papers reported that the anomeric carbon of this sugar characteristically resonates downfield (*ca*. 104.0 ppm) when it is linked to C-4 of D-cymarose, whereas it is observed to resonate upfield (*ca*. 101.8 ppm) when linked to C-4 of D-oleandrose.[3,9] On the basis of the above data, the structure of the pentasaccharide of compounds **40**−**47** was established as *β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Compound 48 (Chain F). NMR data (Tables 1−3) provided evidence that compound **48** was penupogenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside. On the assumption that cymarose was linked at C-3 of the aglycon on the basis of the resonance of C-1′, the determination of the sugar sequence was obtained from a ROESY experiment which showed cross peaks between H-1 of glucose and H-4 of allose and between H-1 of oleandrose and H-4 of cymarose. As pointed out in previous papers[11,13] the sugar sequence of cardiac- and pregnane-type glycosides from Asclepiadaceae show a certain regularity. It should be noted that for all the glycosides isolated from *L. hastata*, the sugar sequences obey the general rule pointed out by Shoji et al.,[13] namely (2,6-dideoxysugars)-*n*−(6-deoxysugars)*n*−glucose linked to the aglycons, respectively.

Antitumoral activities and toxicities have been reported[3,9] for some polyhydroxypregnane glycosides, including examples with a sugar chain of 4−5 residues with different esterified groups on the aglycon. These were examined against a solid-type Ehrlich Carcinoma and Sarcoma 180. On this basis the polyhydroxypregnane glycosides from *L. hastata* possessing a sugar chain with four or five residues and different esterified groups on the aglycon were tested for antitumoral activity in Raji cells (human lymphoblastoid cell line from Burkitt lymphoma).[14] The effect on DNA synthesis and cell cycle distribution in Raji cells of selected glycosides from *L. hastata* having five sugar residues at C-3 is reported in Tables 4 and 5. The chemosensitivity studies were performed in order to correlate different chemical structures of the aglycon with their tumoricidal activity. On the basis of the data in Table 4, it is possible to demonstrate that (a) the presence of an aromatic group in C-12 significantly increases the cytotoxic effect, (b) the presence of an aromatic residue in both C-12 and C-20 amplifies this effect, and (c) the presence of an acetyl group in place of aromatic groups restores the proliferation of the cells. Interestingly, the same compounds which were able to reduce cell proliferation were also able to decrease the percentage of cells

Table 4. Effect of Polyoxypregnane Glycosides from *Leptadenia hastata* on DNA Synthesis in Raji Cells

compds	concentrn (*μ*g/mL)	rel rate of DNA synthesis (%)
none		100
31	0.1	13 ± 8
	0.5	14 ± 9
	0.1	16 ± 5
25	0.1	15 ± 6
	0.5	14 ± 9
	1.0	14 ± 8
43	0.1	16 ± 7
	0.5	16 ± 3
	1.0	15 ± 8
29	0.1	41 ± 17
	0.5	21 ± 10
	1.0	14 ± 8
24	0.1	59 ± 30
	0.5	33 ± 14
	1.0	16 ± 5
26	0.1	98 ± 10
	0.5	83 ± 13
	1.0	69 ± 20
30	0.1	98 ± 10
	0.5	87 ± 10
	1.0	69 ± 20
40	0.1	98 ± 12
	0.5	89 ± 15
	1.0	65 ± 20

Table 5. Effect of Polyoxypregnane Glycosides from *Leptadenia hastata* on Cell Cycle Distribution

compd	concentrn (*γ*/mL)	S phase (%)
31	0.1	5
	0.5	4
	1.0	5
25	0.1	4
	0.5	6
	1.0	4
43	0.1	6
	0.5	5
	1.0	3
29	0.1	22
	0.5	11
	1.0	4
24	0.1	33
	0.5	18
	1.0	10
		4
26	0.1	32
	0.5	30
	1.0	28
30	0.1	30
	0.5	28
	1.0	26
40	0.1	34
	0.5	30
	1.0	18

in the S phase, while for the most part the lymphoblastoid cells were found to be arrested in the G0/G1 phase of the cell cycle (Table 5).

Experimental Section

General Experimental Procedures. A Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D-homonuclear proton chemical shift correlation (COSY), 2D-HOHAHA, $^1H-^{13}C$ HETCOR, and COLOC experiments were obtained as described previously.[1] The ROESY[15] experiments were performed in the phase-sensitive mode (TPPI). The spectral width (t_2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 100, 300, and 500 ms) were acquired in 2 K data points. For processing, a sine bell window function was applied in both dimensions before transformation. Optical rotations were meausured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. Fast atom bombardment mass spectra (FABMS) were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2−6 kV). A AEI MS-30 instrument was used for EIMS. DCCC separations were performed on a Buchi apparatus, equipped with 300 tubes. HPLC separations were performed with a Waters Model 6000A pump equipped with a U6K injector and a Model 401 refractive index detector.

Extraction and Isolation. The plant material and the extraction procedure were previously described.[1] The $CHCl_3$−MeOH (9:1) extract (6.74 g) from *L. hastata* (400 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as the eluent. Fractions (8 mL) were collected and checked by TLC [SiO_2 plates, *n*-BuOH−AcOH−H_2O (12:3:5) and $CHCl_3$/MeOH/H_2O (40:9:1)]. Fractions 47−53 from a Sephadex LH-20 column were directly fractionated by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (4:1) to yield compounds **15** (t_R = 2.5 min, 20.5 mg), **16** (t_R = 7 min, 22 mg) and **17** (t_R = 10 min, 20 mg). Fractions 15−22 (2.83 g) containing the crude glycosidic mixture were purified by DCCC with BuOH−EtOH−AcOH−H_2O (8:4:2:1) in which the stationary phase consisted of the higher phase (ascending mode, flow 10 mL/h) to give two main fractions A (950 mg) and B (1.25 g).

Fraction A was further purified by DCCC [$CHCl_3$−MeOH−H_2O (7:13:8), ascending mode, flow 10 mL/h] to give two fractions A′ (500 mg) and A″ (300 mg) of the less polar glycosides. Final separation was achieved by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (75:25) to yield compounds **18** (t_R = 2.5 min, 22.5 mg), **20** (t_R = 7 min, 36 mg), **21** (t_R = 10 min, 28 mg), **22** (t_R = 12 min, 20 mg), **23** (t_R = 15 min, 15 mg) from fraction A′ as well as **32** (t_R = 12 min, 16 mg), **33** (t_R = 14.5 min, 11 mg), **34** (t_R = 16 min, 13 mg), **35** (t_R = 18 min, 15 mg), **36** (t_R = 11 min, 9 mg), **37** (t_R = 17 min, 7 mg), **38** (t_R = 21 min, 16 mg), **39** (t_R = 17 min, 21 mg), **48** (t_R = 21 min, 23 mg) from fraction A″.

Fraction B was purified by DCCC [$CHCl_3$−H_2O−EtOH−MeOH−*n*-PrOH (9:8:8:6:1), ascending mode, flow 10 mL/h] to give a mixture of the more polar glycosides which were separated by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (7:3) to yield compounds **24** (t_R = 25 min, 70 mg), **25** (t_R = 15 min, 38 mg), **26** (t_R = 18 min, 28 mg), **27** (t_R = 17 min, 19 mg) **28** (t_R = 21 min, 20 mg), **29** (t_R = 17 min, 18 mg), and **30** (t_R = 21 min, 12 mg). Part of the MeOH extract (4 g) was successively chromatographed on a Sephadex LH-20 column, DCCC [BuOH−EtOH−AcOH−H_2O (8:4:2:1)] and HPLC with MeOH−H_2O (65:35) with the above column and elution conditions to give compounds **31** (t_R = 2.5 min, 14.5 mg), **40** (t_R = 7 min, 16 mg), **41** (t_R = 10 min, 58 mg), **42** (t_R = 12 min, 20 mg), **43** (t_R = 15 min, 62 mg), **44** (t_R = 13 min, 60 mg), **45** (t_R = 14.5 min, 11 mg), **46** (t_R = 16 min, 9 mg), and **47** (t_R = 18 min, 25 mg). Compounds **15**−**17** were obtained as crystalline powders and compounds **18**−**48** as amorphous powders.

^{13}C NMR data for the pregnane ester moieties of compounds **15**−**17** and **28**, **39**, **43**, and **47** are given in Table 1; ^1H and ^{13}C NMR data for the sugar moieties of compounds **18**−**48** are in Tables 2 and 3.

Compound 15: $[\alpha]^{25}_D$ +118°; mp = 100−105 °C; EIMS shows no parent peak, *m/z* $(M - C_6H_5COOH)^+$ 494, $(M - \text{cinnamic acid})^+$ 468, $(494 - 2 \times H_2O)^+$ 458, $(468 - 2 \times H_2O)^+$ 432, $(494 - \text{cinnamic acid})^+$ 346, $(346 - 18)^+$ 328, $(328 - 18)^+$ 310, 148 (cinnamic acid), 147, 131 (cinnamoyl cation, base peak), 122 (benzoyl acid), 105 (benzoyl cation); ^1H NMR δ 1.09 (3H, s, Me-19), 1.32 (3H, d, J = 6.6 Hz, Me-21), 1.65 (3H, s, Me-18), 3.10 (1H, br m, H-3), 4.75 (1H, dd, J = 12.0, 4.0 Hz, H-12), 5.03 (1H, q, J = 6.6 Hz, H-20), 5.38 (1H, m, H-6), 6.67 (1H, d, J = 15.9 Hz, H-2′), 7.40 (2H, t, J = 7.6 Hz, H-4″ and H-6″), 7.45 (2H, dd, J = 7.5, 1.5 Hz, H-5′ and H-9′), 7.56 (1H, t, J = 7.4 Hz, H-5″), 7.60 (3H, t, J = 7.6 Hz, H-6′, H-7′, and H-8′), 7.81 (1H, d, J = 15.9 Hz, H-3′), 8.01 (2H, dd, J = 7.4 and 1.2 Hz, H-3″ and H-7″); for ^{13}C NMR see Table 1.

Compound 16: $[\alpha]^{25}_D$ +135.5°; mp = 155−160 °C; EIMS shows no parent peak, *m/z* $(M - C_6H_5COOH)^+$ 485, $(M - \text{nicotinic acid})^+$ 494, $(485 - H_2O)^+$ 467, $(494 - H_2O)^+$ 476, $(467 - \text{nicotinic acid})$ and $(476 - \text{cinnammic acid})^+$ 344, 326, 148, 131, 122 (nicotinic acid, base peak); ^1H NMR δ 1.10 (3H, s, Me-19), 1.31 (3H, d, J = 6.5 Hz, Me-21), 1.67 (3H, s, Me-18), 3.10 (1H, br m, H-3), 4.73 (1H, dd, J = 10.5 and 4.0 Hz, H-12), 4.85 (1H, q, J = 6.5 Hz, H-20), 5.35 (1H, m, H-6), 6.12 (1H, d, J = 15.9 Hz, H-2″), 7.19 (1H, dd, J = 7.8 and 4.9 Hz, H-5′), 7.45 (2H, dd, J = 7.5 and 1.5 Hz, H-5″ and H-9″), 7.50 (1H, d, J = 15.9 Hz, H-3″), 7.60 (3H, t, J = 7.6 Hz, H-6″, H-7″, and H-8″), 8.10 (1H, ddd, J = 7.8, 4.5, and 2.5 Hz, H-4′), 8.72 (1H, dd, J = 4.9, 2.5 Hz, H-6′), 9.18 (1H, d, J = 4.5 Hz, H-2′). For ^{13}C NMR see Table 1.

Compound 17: $[\alpha]^{25}_D$ +112°; mp = 150−155 °C; EIMS shows no parent peak, *m/z* $(M - 45)^+$ 421, $(M - CH_3COOH)^+$ 406, $(421 - 18)^+$ 403, $(406 - 18)^+$ 388, $(M - 2 \times 45)^+$ 376, $(376 - 18)^+$ 358; ^1H NMR δ 1.10 (3H, s, Me-19), 1.30 (3H, d, J = 6.6 Hz, Me-21), 1.65 (3H, s, Me-18), 1.82 and 1.85 (each 3H, s, CO*Me*), 3.10 (1H, br m, H-3), 4.45 (1H, dd, J = 10.5, 4.0 Hz, H-12), 4.65 (1H, q, J = 6.6 Hz, H-20), 5.35 (1H, m, H-6). For ^{13}C NMR see Table 1.

Compound 18: $[\alpha]^{25}_D$ +95°; negative FABMS *m/z* $[M - H]^-$ 943, $[(M - H) - 144]^-$ 799, $[799 - 144]^-$ 655, $[655 - 144]^-$ 511. NMR data for the aglycon moiety

are identical to those reported for compound **8**.[1] For the sugar moiety see Tables 2 and 3.

Compound 19: $[\alpha]^{25}_D$ +103°; negative FABMS *m/z* $[M - H]^-$ 1047, $[(M - H) - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615. NMR data for the aglycon moiety are identical to those for compound **7**.[1] For the sugar moiety see Tables 2 and 3.

Compound **20**: $[\alpha]^{25}_D$ +35°; negative FABMS *m/z* $[M - H]^-$ 1103, $[(M - H) - 160]^-$ 943, $[943 - 144]^-$ 799, $[799 - 144]^-$ 655, $[655 - 144]^-$ 511. NMR data for the aglycon moiety are identical to those for compound **8**.[1] For the sugar moiety see Tables 2 and 3.

Compound 21: $[\alpha]^{25}_D$ +44°; negative FABMS *m/z* $[M - H]^-$ 1207, $[(M - H) - 160]^-$ 1047, $[1047 - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615. NMR data for the aglycon moiety are identical to those for compound **7**.[1] For the sugar moiety see Tables 2 and 3.

Compound 22: $[\alpha]^{25}_D$ +51°; negative FABMS *m/z* $[M - H]^-$ 1181, $[(M - H) - 160]^-$ 1021, $[1021 - 144]^-$ 877, $[877 - 144]^-$ 733, $[733 - 144]^-$ 589. NMR data for the aglycon moiety are superimposable on those for compound **2**.[1] For the sugar moiety see Tables 2 and 3.

Compound 23: $[\alpha]^{25}_D$ +53°; negative FABMS *m/z* $[M - H]^-$ 1145, $[(M - H) - 160]^-$ 985, $[985 - 144]^-$ 841, $[841 - 144]^-$ 697, $[697 - 144]^-$ 553. NMR data for the aglycon moiety are superimposable on those for compound **4**.[1] For the sugar moiety see Tables 2 and 3.

Compound 24: $[\alpha]^{25}_D$ +72°; negative FABMS *m/z* $[M - H]^-$ 1265, $[(M - H) - 162]^-$ 1103, $[1103 - 160]^-$ 943, $[943 - 144]^-$ 799, $[799 - 144]^-$ 655, $[655 - 144]^-$ 511. NMR data for the aglycon moiety are identical to those for compound **8**.[1] For the sugar moiety see Tables 2 and 3.

Compound 25: $[\alpha]^{25}_D$ +81°; negative FABMS *m/z* $[M - H]^-$ 1369, $[(M - H) - 162]^-$ 1207, $[1207 - 160]^-$ 1047, $[1047 - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615. NMR data for the aglycon moiety are identical to those for compound **7**.[1] For the sugar moiety see Tables 2 and 3.

Compound 26: $[\alpha]^{25}_D$ +65°; negative FABMS *m/z* $[M - H]^-$ 1177, $[(M - H) - 162]^-$ 1015, $[1015 - 160]^-$ 855, $[855 - 144]^-$ 711, $[711 - 144]^-$ 567, $[567 - 144]^-$ 423. NMR data for the aglycon moiety are superimposable on those for compound **1**.[1] For the sugar moiety see Tables 2 and 3.

Compound 27: $[\alpha]^{25}_D$ +100.5°; negative FABMS *m/z* $[M - H]^-$ 1282, $[(M - H) - 162]^-$ 1120, $[1120 - 160]^-$ 960, $[960 - 144]^-$ 816, $[816 - 144]^-$ 672, $[672 - 144]^-$ 528. NMR data for the aglycon moiety are superimposable on those for compound **5**.[1] For the sugar moiety see Tables 2 and 3.

Compound 28: $[\alpha]^{25}_D$ +73°; negative FABMS *m/z* $[M - H]^-$ 1239, $[(M - H) - 162]^-$ 1077, $[1077 - 160]^-$ 917, $[917 - 144]^-$ 773, $[773 - 144]^-$ 629, $[629 - 144]^-$ 485; ^{1}H NMR δ 1.11 (3H, d, J = 6.5 Hz, Me-21), 1.20 (3H, s, Me-19), 1.65 (3H, s, Me-18), 3.50 (1H, br m, H-3), 3.88 (1H, q, J = 6.5 Hz, H-20), 4.78 (1H, dd, J = 10.5, 4.5 Hz, H-12), 5.36 (1H, m, H-6), 7.40 (2H, t, J = 7.4 Hz, H-4′ and H-6′), 7.56 (1H, t, J = 7.4 Hz, H-5′), 8.01 (2H, dd, J = 7.4, 1.2 Hz, H-3′, H-7′). For ^{13}C NMR of the aglycon moiety see Table 1. For ^{1}H and ^{13}C NMR of the sugar moiety see Tables 2 and 3.

Compound 29: $[\alpha]^{25}_D$ +133°; negative FABMS *m/z* $[M - H]^-$ 1263, $[(M - H) - 162]^-$ 1101, $[1101 - 160]^-$ 941, $[941 - 144]^-$ 797, $[797 - 144]^-$ 653, $[653 - 144]^-$ 509. NMR data for the aglycon moiety are superimposable on those for kidjolanin **12**.[1] For the sugar moiety see Tables 2 and 3.

Compound 30: $[\alpha]^{25}_D$ +121°; negative FABMS *m/z* $[M - H]^-$ 1175, $[(M - H) - 162]^-$ 1013, $[1013 - 160]^-$ 853, $[853 - 144]^-$ 709, $[709 - 144]^-$ 565, $[565 - 144]^-$ 421. NMR data for the aglycon moiety are superimposable on those for metaplexigenin **13**.[1] For the sugar moiety see Tables 2 and 3.

Compound 31: $[\alpha]^{25}_D$ +97°; negative FABMS *m/z* $[M - H]^-$ 1369, $[(M - H) - 162]^-$ 1207, $[1207 - 160]^-$ 1047, $[1047 - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615. NMR data for the aglycon moiety are identical to those for compound **7**.[1] For the sugar moiety see Tables 2 and 3.

Compound 32: $[\alpha]^{25}_D$ +82; negative FABMS *m/z* $[M - H]^-$ 853, $[(M - H) - 144]^-$ 709, $[709 - 144]^-$ 565, $[565 - 144]^-$ 421. NMR data for the aglycon moiety are superimposable on those for metaplexigenin **13**.[1] For the sugar moiety see Tables 2 and 3.

Compound 33: $[\alpha]^{25}_D$ +90°; negative FABMS *m/z* $[M - H]^-$ 915, $[(M - H) - 144]^-$ 771, $[771 - 144]^-$ 627, $[627 - 144]^-$ 483. NMR data for the aglycon moiety are superimposable on those for cynanforidin **14**.[1] For the sugar moiety see Tables 2 and 3.

Compound 34: $[\alpha]^{25}_D$ +91°; negative FABMS *m/z* $[M - H]^-$ 1207, $[(M - H) - 160]^-$ 1047, $[1047 - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615. NMR data for the aglycon moiety are identical to those for compound **7**.[1] For the sugar moiety see Tables 2 and 3.

Compound 35: $[\alpha]^{25}_D$ +106°; negative FABMS *m/z* $[M - H]^-$ 1181, $[(M- H) - 160]^-$ 1021, $[1021 - 144]^-$ 877, $[877 - 144]^-$ 733, $[733 - 144]^-$ 589. NMR data for the aglycon moiety are superimposable to those for compound **2**.[1] For the sugar moiety see Tables 2 and 3.

Compound 36: $[\alpha]^{25}_D$ +121°; negative FABMS *m/z* $[M - H]^-$ 1208, $[(M - H) - 160]^-$ 1049, $[1049 -144]^-$ 905, $[905 - 144]^-$ 761, $[761 - 144]^-$ 617. NMR data for the aglycon moiety are superimposable to those for gagaminin **11**.[1] For the sugar moiety see Tables 2 and 3.

Compound 37: $[\alpha]^{25}_D$ +132°; negative FABMS *m/z* $[M - H]^-$ 1075, $[(M - H) - 160]^-$ 915, $[915 - 144]^-$ 771, $[771 - 144]^-$ 627, $[627 - 144]^-$ 483. NMR data for the aglycon moiety are superimposable to those for cynanforidin **14**.[1] For the sugar moiety see Tables 2 and 3.

Compound 38: $[\alpha]^{25}_D$ +157°; negative FABMS *m/z* $[M - H]^-$ 1076, $[(M - H) - 160]^-$ 916, $[916 - 144]^-$ 772, $[772 - 144]^-$ 628, $[628 - 144]^-$ 484. NMR data for the aglycon moiety are superimposable to those for compound **6**.[1] For the sugar moiety see Tables 2 and 3.

Compound 39: $[\alpha]^{25}_D$ +100°; negative *m/z* $[M - H]^-$ 1223, $[1223 - 160]^-$ 1063, $[1063 - 144]^-$ 919, $[919 - 144]^-$ 775, $[775 - 144]^-$ 621; ^{1}H NMR for the aglycon δ 1.08 (3H, s, Me-19), 1.32 (3H, d, J = 6.6 Hz, Me-21), 1.67 (3H, s, Me-18), 3.50 (1H, br m, H-3), 4.70 (1H, dd, J = 10.0, 4.0 Hz, H-12), 5.07 (1H, q, J = 6.6 Hz, H-20), 5.35 (1H, m, H-6), 6.67 (1H, d, J = 15.9 Hz, H-2′), 6.80 (1H, br d, H-3″), 7.32 (1H, br m), 7.42 (2H, dd, J = 7.5, 1.5 Hz, H-5′, and H-9′), 7.50 (1H, t, J = 7.4 Hz, H-6″), 7.63 (3H, t, J = 7.6 Hz, H-6′, H-7′, and H-8′), 7.81 (1H, d, J = 15.9 Hz, H-3′), 8.00 (1H, dd, J = 7.4, 1.2 Hz,

H-7″). For ^{13}C NMR of the aglycon moiety see Table 1. For ^{1}H and ^{13}C NMR of the sugar moiety see Tables 2 and 3.

Compound 40: $[\alpha]^{25}_{D}$ +126°; negative FABMS *m/z* $[M - H]^-$ 1177, $[(M - H) - 162]^-$ 1015, $[1015 - 160]^-$ 855, $[855 - 144]^-$ 711, $[711 - 144]^-$ 567, $[567 - 144]^-$ 423. NMR data for the aglycon moiety are superimposable to those for compound **1**.[1] For the sugar moiety see Tables 2 and 3.

Compound 41: $[\alpha]^{25}_{D}$ +133°; negative FABMS *m/z* $[M - H]^-$ 1265, $[(M - H) - 162]^-$ 1103, $[1103 - 160]^-$ 943, $[943 - 144]^-$ 799, $[799 - 144]^-$ 655, $[655 - 144]^-$ 511. NMR data for the aglycon moiety are identical to those for compound **8**.[1] For the sugar moiety see Tables 2 and 3.

Compound 42: $[\alpha]^{25}_{D}$ +160°; negative FABMS *m/z* $[M - H]^-$ 1282, $[(M - H) - 162]^-$ 1120, $[1120 - 160]^-$ 960, $[960 - 144]^-$ 816, $[816 - 144]^-$ 672, $[672 - 144]^-$ 528. NMR data for the aglycon moiety are superimposable to those for compound **5**.[1] For the sugar moiety see Tables 2 and 3.

Compound 43: $[\alpha]^{25}_{D}$ +144°; negative FABMS *m/z* $[M - H]^-$ 1369, $[(M - H) - 162]^-$ 1207, $[1207 - 160]^-$ 1047, $[1047 - 144]^-$ 903, $[903 - 144]^-$ 759, $[759 - 144]^-$ 615; ^{1}H NMR δ 1.10 (3H, s, Me-19), 1.32 (3H, d, J = 6.6 Hz, Me-21), 1.67 (3H, s, Me-18), 3.51 (1H, br m, H-3), 4.70 (1H, dd, J = 10.5, 4.0 Hz, H-12), 5.03 (1H, q, J = 6.6 Hz, H-20), 5.36 (1H, m, H-6), 6.67 (1H, d, J = 15.9 Hz, H-2′), 7.40 (2H, t, J = 7.6 Hz, H-4″ and H-6″), 7.45 (2H, dd, J = 7.5, 1.5 Hz, H-5′, and H-9′), 7.56 (1H, t, J = 7.4 Hz, H-5″), 7.60 (3H, t, J = 7.6 Hz, H-6′, H-7′, and H-8′), 7.81 (1H, d, J = 15.9 Hz, H-3′), 8.01 (2H, dd, J = 7.4, 1.2 Hz, H-3″, and H-7″). For ^{13}C NMR of the aglycon moiety see Table 1. For ^{1}H and ^{13}C NMR of the sugar moiety see Tables 2 and 3.

Compound 44: $[\alpha]^{25}_{D}$ +164°; negative FABMS *m/z* $[M - H]^-$ 1237, $[(M - H) - 162]^-$ 1075, $[1075 - 160]^-$ 915, $[915 - 144]^-$ 771, $[771 - 144]^-$ 627, $[627 - 144]^-$ 483. NMR data for the aglycon moiety are superimposable to those for cynanforidin **14**.[1] For the sugar moiety see Tables 2 and 3.

Compound 45: $[\alpha]^{25}_{D}$ +187°; negative FABMS *m/z* $[M - H]^-$ 1175, $[(M - H) - 162]^-$ 1013, $[1013 - 160]^-$ 853, $[853 - 144]^-$ 709, $[709 - 144]^-$ 565, $[565 - 144]^-$ 421. NMR data for the aglycon moiety are superimposable to those for metaplexigenin **13**.[1] For the sugar moiety see Tables 2 and 3.

Compound 46: $[\alpha]^{25}_{D}$ +193°; negative FABMS *m/z* $[M - H]^-$ 1263, $[(M - H) - 162]^-$ 1101, $[1101 - 160]^-$ 941, $[941 - 144]^-$ 797, $[797 - 144]^-$ 653, $[653 - 144]^-$ 509. NMR data for the aglycon moiety are superimposable to those for kidjolanin **12**.[1] For the sugar moiety see Tables 2 and 3.

Compound 47: $[\alpha]^{25}_{D}$ +135.5°; negative FABMS *m/z* $[M - H]^-$ 1412, $[(M - H) - Ac]^-$ 1370, $[(M - H) - 162]^-$ 1250, $[1250 - 160]^-$ 1090, $[1090 - 144]^-$ 946, $[946 - 144]^-$ 802, $[802 - 144]^-$ 658; ^{1}H NMR for the aglycon moiety δ 1.10 (3H, s, Me-19), 1.30 (3H, d, J = 6.5 Hz, Me-21), 1.67 (3H, s, Me-18), 1.82 (3H, s, CO*Me*), 3.51 (1H, br m, H-3), 4.70 (1H, dd, J = 10.5, 4.0 Hz, H-12), 4.80 (1H, q, J = 6.5 Hz, H-20), 5.38 (1H, m, H-6), 6.12 (1H, d, J = 15.9 Hz, H-2″), 7.19 (1H, dd, J = 7.8, 4.9 Hz, H-5′), 7.45 (2H, dd, J = 7.5, 1.5 Hz, H-5″, and H-9″), 7.50 (1H, d, J = 15.9 Hz, H-3″), 7.60 (3H, t, J = 7.6 Hz, H-6″, H-7″, and H-8″), 8.10 (1H, ddd, J = 7.8, 4.5, 2.5 Hz, H-4′), 8.72 (1H, dd, J = 4.9, 2.5 Hz, H-6′), 9.18 (1H d, J = 4.5 Hz, H-2′). For ^{13}C NMR of the aglycon moiety see Table 1. For ^{1}H and ^{13}C NMR of the sugar moiety see Tables 2 and 3.

Compound 48: $[\alpha]^{25}_{D}$ +120°; negative FABMS *m/z* $[M - H]^-$ 1131, $[(M - H) - 162]^-$ 969, $[969 - 160]^-$ 809, $[809 - 144]^-$ 655, $[655 - 144]^-$ 511; NMR data for the aglycon moiety are identical to those for compound **8**.[1] For the sugar moiety see Tables 2 and 3.

Biological Assay.[14] Raji cells (human lymphoblastoid cell line from Burkitt lymphoma) were grown in suspension in RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cells were maintained in logarithmic growth in a humidified incubator at 37 °C with 5% CO_2 and subcultured twice weekly, depending on their doubling time. For experimental use exponentially growing cells were plated at a density of 3×10^5 cells/well in 96-well flat bottom microplates. Cells were cultured in the presence or absence of the different molecules and allowed to grow for 24 h.

DNA synthesis was determined by adding to the cultures 0.5 μCi of [methyl-^{3}H]thymidine (41Ci/mmol). After 6 h incubation, the cells were collected by a cell harvester onto glass fiber filters. The filter disks were dried and placed in scintillation fluid to determine the amount of radioactivity incorporated into the DNA. For flow cytometric analysis the nuclei of the lymphoblastoid cells (106 cells/mL) were stained with the fluorescent dye propidium iodide. The stained cells were subjected to flow cytometry (FACS IV cell sorter).

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NP960251E