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NEW POLYOXYPREGNANE ESTER DERIVATIVES FROM *LEPTADENIA HASTATA*

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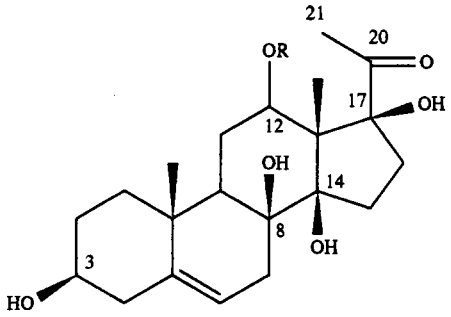
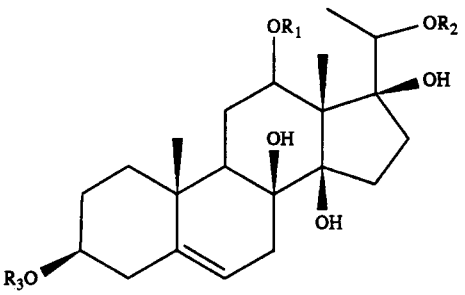
ABSTRACT.—Six new polyoxypregnane esters [**1–6**] having sarcostin or deacetylmetaplexigenin as the aglycones, and acetyl, benzoyl, cinnamoyl, and nicotinoyl residues as the ester moieties linked at C-12 and/or at C-20, were isolated from *Leptadenia hastata*. In addition, three new glycosides [**7–9**] with D-cymarose and D-oleandrose linked at C-3 were present in the $CHCl_3$ extract. Structures were deduced on the basis of spectral evidence.

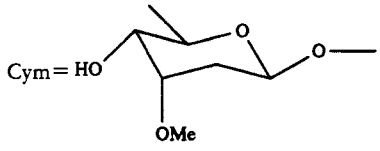
As a part of our studies on the biologically active metabolites from Senegalese medicinal plants, we describe herein the separation and structural elucidation of six new polyoxypregnane esters [**1–6**] and three new glycosides [**7–9**], isolated together with five known esters [**10–14**] from the $CHCl_3$ extract of the bark of *Leptadenia hastata* Decne. (Asclepiadaceae). The bark of this plant is used as an anti-inflammatory and antitumor drug in Senegalese folk medicine (1). To our knowledge, no phytochemical

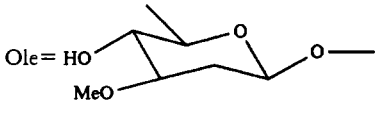




6 R=Nic
12 R=Cinn
13 R=Ac
14 RBz

	R_1	R_2	R_3
1	Ac	H	H
2	Bz	Bz	H
3	Bz	Cinn	H
4	Cinn	Ac	H
5	Nic	Ac	H
7	Bz	Cinn	Cym
8	Cinn	H	Ole-(1→4)-Cym—
9	Bz	Cinn	Ole-(1→4)-Cym—
10	Cinn	H	H
11	Cinn	Nic	H





Ac=Acetyl; Bz=Benzoyl; Cinn=Cinnamoyl; Nic=Nicotinoyl

work has been reported on this species. The plants of the family Asclepiadaceae are known to contain a number of C/D-*cis*-polyoxypregnane ester derivatives possessing an oxo or a carbinol group at C-20 (2), and some of these glycosidic derivatives have shown antitumor activity against Ehrlich carcinoma and melanoma B-16 (3). Cytotoxicity has also been reported for other pregnane derivatives (4). Therefore, it appeared of interest to investigate the components of an extract of *L. hastata*.

RESULTS AND DISCUSSION

The $CHCl_3$ extract of bark from *L. hastata* afforded two main fractions containing mixtures of polyoxypregnane ester derivatives that were separated by SiO_2 cc. The novel esters **1–6** as well as the known esters 12-*O*-cinnamoylsarcostin (penupogenin) [**10**] (5), 12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin (gagaminin) [**11**] (6), 12-*O*-cinnamoyldeacetylmetaplexigenin (kidjolanin) [**12**] (5), metaplexigenin [**13**] (7), and 12-*O*-benzoyldeacetylmetaplexigenin (cynanforidin) [**14**] (8) were isolated from the less polar fraction. The glycosides **7–9** were purified from the more polar fraction by reversed-phase hplc.

Ms and nmr data of compounds **1–5** (Table 1 and Experimental) suggested that they were sarcostin (3β,8β,12β,14β,17β,20-hexahydroxypregn-5-ene) ester derivatives (5,8). The ^{13}C-nmr spectra of **1–5** exhibited signals consistent with the presence of one or two acid moieties and twenty-one carbon signals. Six of these were oxygenated and were assignable to three secondary hydroxyl groups (C-3, C-12, and C-20) and three tertiary hydroxyl groups (C-8, C-14, and C-17) of a C/D-*cis*-pregnane system (8). The ^{1}H-nmr spectra of **1–5** indicated an olefinic proton signal at ca. δ 5.35 (1H, m), correlated by ^{1}H-^{13}C HETCOR nmr to carbon signals at 119.4 ppm (H-6) and linked to a quaternary carbon resonating at 140.1 ppm (C-5) to support the presence of Δ^5 unsaturation, as well as one doublet and two singlet methyl proton signals to complete the polyoxypregnane structure. A secondary hydroxyl group was assigned at the 3α position of **1–5** by the signal pattern of the methine proton at δ 3.15 and by the chemical shift of the correlated carbon signal (72.54 ppm, CH-3). Three tertiary hydroxyl groups were placed at the C-8 (75.0 ppm), C-14 (88.58 ppm), and C-17 β (89.90 ppm) positions by biogenetic analogy to other Asclepiadaceous aglycones and by comparison with model compounds (9).

The ^{1}H-nmr spectrum of compound **1** (molecular formula $C_{23}H_{36}O_7$ by DEPT, ^{13}C-nmr, and ms analysis) showed an alcoholic methine proton signal at δ 3.88 (q, J=6.6 Hz) that was correlated by a ^{1}H-^{13}C HETCOR nmr experiment to the carbon signal at 71.83 ppm (CH), attributed to C-20 of sarcostin, and an esterified methine proton at δ 4.42 (dd, J=12 and 4 Hz) correlated to the carbon signal at 73.60 ppm (CH) assigned to position 12 of sarcostin by its chemical shift and coupling pattern. The ester linked at C-12 was determined to be an acetyl as shown by nmr data (^{1}H δ 1.92, ^{13}C δ 170.20 and 20.61). Furthermore, the Me-21 signal (d, J=6.6 Hz) resonated at δ 1.12 and correlated with the Me signal at 18.72 ppm, confirming that C-20 was not esterified. Compound **1** was thus concluded to be 12-*O*-acetylsarcostin.

Compound **2** was determined to have the molecular formula $C_{35}H_{42}O_8$. Its ^{1}H-nmr spectrum showed two Me singlet signals at δ 1.65 (Me-18) and 1.08 (Me-19) that were almost superimposable with those of **1**, and a Me doublet signal (Me-21) shifted downfield from δ 1.12 to δ 1.32. Two esterified methine proton signals at δ 4.77 (dd, J=10 and 4 Hz) and at δ 5.05 (q, J=6.6 Hz) correlating to ^{13}C-nmr signals at 76.00 and 75.05 ppm (both CH) by HETCOR were consistent with a sarcostin derivative esterified at positions 12 and 20.

The ester moieties were two benzoyl groups as shown by the ^{1}H- [δ 8.01 (4H, dd,

TABLE 1. ^{13}C-Nmr Data for Compounds **1–8** (500 MHz in CD_3OD).[a]

Carbon	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C-1	39.78	39.79	39.78	39.82	39.75	39.54	38.61	38.74
C-2	31.73	31.68	31.74	31.19	31.73	30.61	30.61	31.74
C-3	72.54	72.30	72.54	71.70	72.40	72.32	79.17	79.26
C-4	42.93	43.00	42.92	42.50	42.95	42.51	37.90	38.03
C-5	140.09	140.09	140.05	140.02	140.05	141.00	139.90	140.00
C-6	119.31	119.86	119.32	120.08	118.80	118.63	119.68	119.74
C-7	34.28	34.52	34.33	34.54	33.12	34.88	34.34	34.50
C-8	75.02	75.03	75.03	75.31	75.02	74.60	75.00	75.03
C-9	44.67	44.67	44.70	44.76	45.22	44.80	44.61	44.65
C-10	36.80	37.50	36.74	37.90	36.70	37.86	38.01	38.02
C-11	25.00	26.00	26.20	26.10	25.00	25.60	25.96	26.05
C-12	73.60	76.00	75.41	76.27	73.57	74.11	76.27	76.32
C-13	56.10	57.70	57.70	57.10	56.14	56.57	57.20	57.23
C-14	88.58	88.60	88.54	88.59	88.59	89.72	88.40	88.52
C-15	35.52	35.18	35.17	35.22	35.22	33.30	35.04	35.12
C-16	34.10	34.08	34.03	34.17	34.07	31.61	34.01	34.22
C-17	89.90	89.62	89.60	89.95	89.65	92.69	89.47	89.90
C-18	11.35	11.31	11.30	11.32	11.32	10.95	11.15	11.32
C-19	18.30	18.58	18.35	18.30	18.28	18.34	18.28	18.28
C-20	71.83	74.20	75.30	75.03	75.20	209.00	74.83	71.79
C-21	18.72	15.24	15.25	15.00	15.25	27.55	15.25	18.68
CH_3CO	178.00			178.20	178.00			
CH_3CO	21.00			22.00	21.50			
C-1′		168.00	168.30	167.10			168.50	167.15
C-2′		130.90	131.00	118.00	153.75	153.70	131.10	118.45
C-3′		129.80	129.75	146.14	127.25	127.15	129.90	146.99
C-4′		130.00	130.15	135.45	136.45	136.30	130.20	135.00
C-5′		134.10	134.34	129.70	123.70	123.70	134.52	129.10
C-6′		130.00	130.15	128.99	153.75	153.70	130.20	128.76
C-7′		129.80	129.75	131.11	164.80	164.75	129.90	131.67
C-8′				128.99				128.76
C-9′				129.70				129.10
C-1″		168.10	167.00				167.15	
C-2″		131.00	118.97				118.90	
C-3″		129.70	146.14				146.15	
C-4″		130.25	135.00				136.00	
C-5″		134.10	129.50				128.90	
C-6″		130.25	128.91				128.70	
C-7″		129.70	131.10				131.71	
C-8″			128.91				128.70	
C-9″			129.50				128.90	

[a]Assignments confirmed by DEPT, HETCOR, and COLOC experiments.

J=7.4 and 1.2 Hz), 7.40 (4H, t, J=7.4 Hz), 7.52 (2H, t, J=7.4 Hz) and ^{13}C-nmr spectra. The ester linkage at the C-20 position led to the downfield shift (β-effect) by 3.80 ppm observed for the C-20 resonance [75.20 ppm in **2** against 71.83 ppm in **1** and the upfield shift (γ-effect) experienced by the C-21 resonance (15.24 ppm in **2** compared to 18.72 ppm in **1**)]. It has been noted that when a benzoyl occurs instead of an acetyl as the ester moiety, a significant downfield shift of the esterified proton occurs (ca. 0.35 ppm for H-12 in **2** with respect to H-12 in **1**). Compound **2** was thus deduced as 12,20-*O*-dibenzoylsarcostin.

Compound **3** has the molecular formula $C_{37}H_{44}O_8$. Spectral data indicated this

compound to be a diester of sarcostin with benzoyl and *trans*-cinnamoyl moieties, and C-12 and C-20 as the esterification sites (Table 1). The pregnane system of **3** gave almost identical ^{1}H- and ^{13}C-nmr spectra to **2** with the exception of H-20 which was observed to resonate upfield (δ 4.85 in **3** against δ 5.05 in **2**). It only remained to establish the relative positions at C-20 and at C-12 of the cinnamoyl and benzoyl residues. In order to confirm the position of the ester linkages, a COLOC (2D ^{1}H-^{13}C shift correlation spectrum by long-range coupling) nmr experiment was carried out. Three clear long-range carbon-proton shift correlations between the carbonyl carbon at 167.00 ppm and H-2″ (δ 6.12, d, J=15.9 Hz) and H-3″ (δ 7.50, d, J=15.9 Hz), and both of the cinnamoyl moieties as well as H-20 (δ 4.85, q, J=6.5 Hz) of the pregnane moiety, were observed in the COLOC spectrum. Therefore, the cinnamoyl group is esterified at position 20, and consequently the benzoyl group is linked at C-12. Compound **3** was thus deduced as 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin.

A comparison of the spectra of 20-*O*-cinnamoyl derivatives like compound **3** and 12-*O*-cinnamoyl derivatives like penupogenin [**10**] and gagaminin [**11**] led us to note some diagnostic differences in the resonances of H-3″ and H-2″ of *trans*-cinnamic acid when it is linked at C-20 or C-12 of sarcostin. In particular, H-2″ was observed to resonate at δ 6.12 and H-3″ at δ 7.50 in C-20 derivatives, whereas they resonate at lower field (ca. δ 6.67 and 7.81, respectively) in C-12 ester derivatives.

From ms and nmr data, compound **4** was deduced as 12-*O*-cinnamoyl-20-*O*-acetylsarcostin and **5** as 12-*O*-nicotinoyl-20-*O*-acetylsarcostin. Compounds **4** ($C_{30}H_{40}O_8$) and **5** ($C_{29}H_{39}O_8N$) gave spectral evidence indicating that they were diesters of sarcostin with *trans*-cinnamic and acetic acid in **4** and nicotinic acid [^{1}H nmr, δ 7.19 (1H, dd, J=7.8 and 4.9 Hz, H-5′), 8.10 (1H, ddd, J=7.8, 4.5, and 2.5 Hz, H-4′), 8.72 (1H, dd, J=4.9 and 2.5 Hz, H-6′), 9.18 (1H, d, J=4.5 Hz, H-2′)] and acetic acid in **5**, linked at C-12 and/or at C-20 of the aglycone. The chemical shifts of H-2′ and H-3′ (δ 6.67 and 7.81, respectively) of the cinnamoyl moiety in **4**, resonating at significantly lower field with respect to the same signals in **3**, gave evidence of a 12-*O*-cinnamoyl ester like in **10** and **11**. In addition, the resonance of H-20 (δ 4.65) in **4** and **5**, at higher field with respect to H-20 in **2** (δ 5.05) and **3** (δ 4.85), indicated the presence of the acetyl moiety at C-20.

By comparison with previously reported compounds and literature data, compound **6** ($C_{27}H_{35}O_7N$) was deduced to be an ester derivative of deacetylmetaplexigenin (3β,8β,12β,14β,17β-pentahydroxypregn-5-en-20-one) (5). The ester linkage occurs at the C-12β hydroxyl group on the basis of the chemical shift and coupling pattern of the proton at δ 4.86 (H-12α) and its correlated carbon (74.11 ppm). The ester group was assigned as nicotinic acid by its ^{1}H- and ^{13}C-nmr spectra (Table 1).

Glycosides **7–9** have molecular formulas of $C_{44}H_{56}O_{11}$, $C_{44}H_{64}O_{13}$, and $C_{51}H_{68}O_{14}$, respectively, as deduced by DEPT, ^{13}C-nmr, and fabms analysis in the negative-ion mode. Their fabms spectra showed quasi-molecular anions at *m/z* 759, 799, and 903 $[M-H]^-$ and prominent fragment peaks at *m/z* 615 $[m/z\ 759-144]^-$, 511 $[m/z\ 799-(2\times144)]^-$, and 615 $[m/z\ 903-(2\times144)]^-$, respectively, arising from the loss of one or two 3-*O*-methyl-2,6-dideoxyhexose units. The glycosidic linkages were located at the C-3 position of the aglycones by the glycosylation shifts observed for the C-2 (ca. −1.1 ppm), C-3 (ca. +7.0 ppm), and C-4 (ca. −4.5 ppm) resonances in the ^{13}C-nmr spectrum of **7–9** with respect to compounds **1–5**. From the resonances and coupling patterns of the other hydrogen and carbon signals, the aglycone moiety of **7** and **9** was presumed to be 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin [**3**] and the aglycone moiety of **8**, 12-*O*-cinnamoylsarcostin (penupogenin [**10**]).

The ^{1}H-nmr spectrum (500 MHz) for the sugar portion of **7** showed well-resolved

signals for an *O*-methyl group (δ 3.45), a secondary methyl group of a 6-deoxypyranose (δ 1.15, d, J=6.5 Hz), and an anomeric proton (δ 4.82, dd, J=9.6 and 2.0 Hz), whereas methylene protons, ascribable to a 2-deoxy sugar, appeared as overlapped signals in the region 1.55 and 2.08 ppm. These data suggested the sugar unit to be a monomethoxy-2,6-dideoxyhexose. A ^{1}H-^{1}H COSY nmr spectrum led to the sequential assignments (reported in Table 2) within this sugar fragment starting from the anomeric signal. The ^{1}H-^{13}C HETCOR nmr technique correlated all proton signals (from H-1 to H-6) to the corresponding carbon signals. Chemical shifts, the multiplicity of the signals, and values of the coupling constants in the ^{1}H-nmr spectrum as well as ^{13}C-nmr data led to the identification of the sugar as β-D-cymaropyranoside (8,10,11). The large coupling constant (J=9.6 Hz) of the anomeric proton was typical of the axial proton of a 2-deoxyhexose (pyran form), confirming that cymarose is present in the $^4C_1$ (D) conformation and joined to the aglycone through a β-glycosidic linkage (12).

Compounds **8** and **9** possessed the same disaccharide chain, as revealed by their nmr spectra (two OMe proton signals at δ 3.44 and 3.46 and two Me doublet signals at δ 1.18 and 1.28). The β-linkages of the two sugars were shown by the coupling constants of two anomeric proton signals (δ 4.90, dd, J=9.6 and 2.0 Hz; δ 4.55, dd, J=9.1 and 1.5 Hz). The structure of the disaccharide was determined by 2D nmr spectroscopy. The HOHAHA nmr spectrum resolved the overlapped sugar signals into a subset of two monosaccharide spectra while the COSY nmr experiment permitted the sequential assignments within each sugar fragment from H-1 to H-6 (Table 2). The above data and the HETCOR results led to the identification of a terminal β-D-oleandropyranoside linked at C-4 of a β-D-cymaropyranoside (8,10). In fact, C-4 of the cymarose unit was shifted downfield (β-effect) by +8.95 ppm with respect to C-4 in compound **7** as expected for a glycosylation shift. All these data indicated that **7** was 3-*O*-β-D-

TABLE 2. Nmr Data for the Sugar Moieties of Compounds **7–9**[a] (500 MHz in CD_3OD).

		Compound			
		7		**8,9**	
	Position	δ_C	δ_H	δ_C	δ_H
D-Cym	1	97.08	4.82, dd, J=9.6 and 2 Hz	97.01	4.90, dd, J=9.6 and 2.0 Hz
	2	35.90	2.20[b]	36.57	2.08[b]
			1.63[b]		1.55[b]
	3	78.80	3.66, q, J=3.0 Hz	78.40	3.88, q, J=3.0 Hz
	4	74.83	3.20, dd, J=9.5 and 3.0 Hz	83.78	3.30, dd, J=9.5 and 3.0 Hz
	5	69.93	3.80, dq, J=9.5 and 6.4 Hz	69.83	3.90, dq, J=9.5 and 6.4 Hz
	6	18.24	1.15, d, J=6.4	18.28	1.18, d, J=6.4
	3-OMe	57.62	3.45, s	57.26	3.44, s
D-Ole	1			102.65	4.55, dd, J=9.1 and 1.5
	2			37.33	2.35[b]
					1.45[b]
	3			81.49	3.28[b]
	4			76.85	3.18, t, J=9.5 Hz
	5			73.13	3.50, dq, J=9.5 and 6.2 Hz
	6			18.28	1.28, d, J=6.2
	3-OMe			58.42	3.46, s

[a]Assignments confirmed by COSY and HOHAHA experiments.
[b]Overlapped with other signals.

cymaropyranosyl-12-*O*-benzoyl-20-*O*-cinnamoylsarcostin, **8** was 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosylpenupogenin and **9** was 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-12-*O*-benzoyl-20-*O*-cinnamoylsarcostin.

All isolated compounds were tested for their cytotoxic activity on Raji cells, but were shown to be inactive at the doses at which evaluated (see Experimental).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A Bruker WH-250 Spectroscopin or a Bruker AMX-500 spectrometer, equipped with a Bruker X-32 computer using the UXNMR software package, was used for nmr experiments. 2D homonuclear proton chemical shift correlation (COSY) nmr experiments were measured by employing the conventional pulse sequence. The 2D HOHAHA nmr experiments were performed in the phase-sensitive mode (TPPI) (13). The HETCOR and COLOC nmr experiments were performed using CH couplings of 135 Hz and 6 Hz, respectively. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. 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). Hplc separations were performed on a Waters model 6000A pump equipped with a U6K injector and E401 refractive index detector.

PLANT MATERIAL.—The bark of *Leptadenia hastata* Decne (Asclepiadaceae) was collected in Dakar, Senegal, in October 1990, and identified by Prof. E. Bassene. A voucher sample is deposited at the Herbarium of the Faculté Mixte de Medicine et Pharmacie, Laboratoire de Pharmacognosie, Université de Dakar, Senegal.

EXTRACTION AND ISOLATION.—The dried bark (400 g) was successively extracted in a Soxhlet apparatus with petroleum ether and $CHCl_3$ to give 2.8 and 7.8 g of residue, respectively. The $CHCl_3$ residue was chromatographed on a SiO_2 column (400 g) using $CHCl_3$ with an increasing amount of MeOH up to 20%. After being monitored [tlc on SiO_2 plates, $CHCl_3$-MeOH (85:15) and $CHCl_3$-MeOH-H_2O (40:9:1)], fractions were pooled to give the combined fractions A (400 mg), B (3.0 g), and C (300 mg). Fraction B was rechromatographed on another SiO_2 column using a gradient solvent system of $CHCl_3$/MeOH from 10 to 50% to obtain four main fractions: D (500 mg), E (450 mg), F (300 mg), and G (80 mg). Final separation of fractions D–G was achieved by reversed-phase hplc on a C_{18} μ-Bondapak column (30 cm×7.8 mm, flow rate 2.5 ml/min) to afford compounds **13** (R_t 2.5 min, 45 mg), **14** (R_t 7 min, 32 mg), **6** (R_t 10 min, 7 mg), and **12** (R_t 12 min, 71 mg) from fraction D with MeOH-H_2O (85:15) as eluent; **5** (R_t 12 min, 10 mg), **11** (R_t 14.5 min, 7 mg), **2** (R_t 16 min, 52 mg), and **3** (R_t 18 min, 48 mg) from fraction E with MeOH-H_2O (39:11); **1** (R_t 11 min, 35 mg), **10** (R_t 17 min, 55 mg), and **4** (R_t 21 min, 40 mg) from fraction F with MeOH-H_2O (4:1), and **7** (R_t 25 min, 28 mg) from fraction G with MeOH-H_2O (3:1). Fraction C was purified by reversed-phase hplc with the above column and elution conditions with MeOH-H_2O (3:1) to give **8** (R_t 15 min, 20 mg) and **9** (R_t 18 min, 22 mg).

Compounds **10–14** were identified by comparison with literature data (5–8). ^{13}C-Nmr data of the pregnane ester moieties of compounds **1–8** are shown in Table 1; ^{1}H- and ^{13}C-nmr data of the sugar moieties of **7–9** are shown in Table 2.

12-O-*Acetylsarcostin* [**1**].—Mp 150–155°; $[\alpha]^{25}D$ +110° (*c*=1, MeOH); eims *m/z* no parent peak, $(M-H_2O)^+$ 406, $(M-2\times H_2O)^+$ 388, $(M-45)^+$ 379, $(M-CH_3COOH)^+$ 364, (*m/z* 364−18)$^+$ 346, (*m/z* 346−18)$^+$ 328, (*m/z* 328−18)$^+$ 310; ^{1}H nmr δ 1.12 (3H, d, *J*=6.5 Hz, Me-21), 1.20 (3H, s, Me-19), 1.65 (3H, s, Me-18), 1.82 (3H, s, CO*Me*), 3.10 (1H, br m, H-3), 3.88 (1H, q, *J*=6.5 Hz, H-20), 4.42 (1H, dd, *J*=10 and 4 Hz, H-12), 5.38 (1H, m, H-6).

12,20-O-*Dibenzoylsarcostin* [**2**].—Mp 95–100°; $[\alpha]^{25}D$ +121° (*c*=1, MeOH); eims *m/z* no parent peak, $(M-C_6H_5COOH)^+$ 468, (*m/z* 468−H_2O)$^+$ 450, (*m/z* 450−H_2O)$^+$ 432, $(M-2\times C_6H_5COOH)^+$ 346, (*m/z* 346−18)$^+$ 328, (328−18)$^+$ 310, 122 (C_6H_5COOH), 105 (benzoyl cation, base peak); ^{1}H nmr δ 1.08 (3H, s, Me-19), 1.32 (3H, d, *J*=6.5 Hz, Me-21), 1.65 (3H, s, Me-18), 3.15 (1H, br m, H-3), 4.77 (1H, dd, *J*=10.5 and 4.5 Hz, H-12), 5.05 (1H, q, *J*=6.5 Hz, H-20), 5.35 (1H, m, H-6), 7.40 (4H, t, *J*=7.4 Hz, H-4′, H-6′, H-4″, and H-6″), 7.56 (2H, t, *J*=7.4 Hz, H-5′ and H-5″), 8.01 (4H, dd, *J*=7.4 and 1.2 Hz, H-3′, H-7′, H-3″, and H-7″).

12-O-*Benzoyl-20*-O-*cinnamoylsarcostin* [**3**].—Mp 100–105°; $[\alpha]^{25}D$ +128° (*c*=1, MeOH); eims *m/z* no parent peak, $(M-C_6H_5COOH)^+$ 494, (*m/z* 494−H_2O)$^+$ 476, (M−cinnamic acid)$^+$ 468, (*m/z* 494−2×H_2O)$^+$ 458, (468−H_2O)$^+$ 450, (*m/z* 468−2×H_2O)$^+$ 432, (*m/z* 494−cinnamic acid)$^+$ 346, (*m/z* 346−18)$^+$ 328, (*m/z* 328−18)$^+$ 310, 148 (cinnamic acid), 147, 131 (cinnamoyl cation, base peak); ^{1}H nmr δ 1.10 (3H, s, Me-19), 1.35 (3H, d, *J*=6.6 Hz, Me-21), 1.70 (3H, s, Me-18), 3.15 (1H, br m, H-3), 4.77 (1H, dd, *J*=10.5

and 4.5 Hz, H-12), 4.85 (1H, q, J=6.6 Hz, H-20), 5.36 (1H, m, H-6), 6.12 (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 and 1.5 Hz, H-5″ and H-9″), 7.50 (1H, d, J=15.9 Hz, H-3″), 7.56 (1H, t, J=7.4 Hz, H-5′), 7.60 (1H, t, J=7.6 Hz, H-6″, H-7″, and H-8″), 8.01 (2H, dd, J=7.4 and 1.2 Hz, H-3′ and H-7′).

12-O-Cinnamoyl-20-O-acetylsarcostin [**4**].—Mp 135–140°; $[\alpha]^{25}D$ +130.5° (c=1, MeOH); eims *m/z* no parent peak $(M-CH_3COOH)^+$ 468, $(m/z\ 468-H_2O)^+$ 450, $(M-C_6H_5COOH)^+$ 406, $(m/z\ 406-H_2O)^+$ 388, $(m/z\ 406-CH_3COOH)^+$ 346, 122 (benzoic acid), 105 (benzoyl cation, base peak); ^1H nmr δ 1.10 (3H, s, Me-19), 1.30 (3H, d, J=6.5 Hz, Me-21), 1.65 (3H, s, Me-18), 1.82 (3H, s, COMe), 3.10 (1H, br m, H-3), 4.65 (1H, q, J=6.5 Hz, H-20), 4.75 (1H, dd, J=10 and 4 Hz, H-12), 5.35 (1H, m, H-6), 6.67 (1H, d, J=16.0 Hz, H-2′), 7.42 (2H, dd, J=7.5 and 1.5 Hz, H-5′ and H-9′), 7.63 (3H, t, J=7.5 Hz, H-6′, H-7′, and H-8′), 7.81 (1H, d, J=16 Hz, H-3′).

12-O-Nicotinyl-20-O-acetylsarcostin [**5**].—Mp 155–160°; $[\alpha]^{25}D$ +145.5° (c=1, MeOH); eims *m/z* no parent peak, $(M-CH_3COOH)^+$ 469, $(m/z\ 469-H_2O)^+$ 451, $(M-$nicotinic acid$)^+$ 406, $(m/z\ 406-H_2O)^+$ 388, $(m/z\ 388-CH_3COOH)^+$ 328, $(m/z\ 328-18)^+$ 310, 122 (nicotinic acid, base peak); ^1H nmr δ 1.10 (3H, s, Me-19), 1.31 (3H, d, J=6.5 Hz, Me-21), 1.65 (3H, s, Me-18), 1.82 (3H, s, COMe), 3.14 (1H, br m, H-3), 4.65 (1H, q, J=6.5 Hz, H-20), 4.73 (1H, dd, J=10 and 4 Hz, H-12), 5.34 (1H, m, H-6), 7.19 (1H, dd, J=7.8 and 4.9 Hz, H-5′), 8.10 (1H, ddd, J=7.8, 4.5, and 2.5 Hz, H-4′), 8.72 (1H, dd, J=4.9 and 2.5 Hz, H-6′), 9.18 (1H, d, J=4.5 Hz, H-2′).

12-O-Nicotinoyldeactylmetaplexigenin [**6**].—Mp 198–203°; $[\alpha]^{25}D$ +178° (c=1, MeOH); eims *m/z* no parent peak, $(M-$nicotinic acid$)^+$ 378, $(m/z\ 378-H_2O)^+$ 360, $(m/z\ 360-2\times H_2O)^+$ 342, 122 (nicotinic acid, base peak); ^1H nmr δ 1.20 (3H, s, Me-19), 1.62 (3H, s, Me-18), 2.28 (3H, s, Me-21), 1.70 and 2.00 (H_2-15), 1.90 and 2.88 (H_2-16), 3.14 (1H, br m, H-3), 4.68 (1H, dd, J=10 and 4 Hz, H-12), 5.34 (1H, m, H-6), 7.18 (1H, dd, J=7.8 and 4.9 Hz, H-5′), 8.09 (1H, ddd, J= 7.8, 4.5, and 2.5 Hz, H-4′), 8.72 (1H, dd, J=4.9 and 2.5 Hz, H-6′), 9.16 (1H, d, J=4.5 Hz, H-2′).

3-O-β-D-Cymaropyranosyl-12-O-benzoyl-20-O-cinnamoylsarcostin [**7**].—$[\alpha]^{25}D$ +73° (c=1, MeOH); fabms, see text.

3-O-β-D-Oleandropyranosyl-(1→4)-β-D-cymaropyranosylpenupogenin [**8**].—$[\alpha]^{25}D$ +80° (c=1, MeOH); fabms, see text; ^1H nmr δ 1.12 (3H, d, J=6.4 Hz, Me-21), 1.20 (3H, s, Me-19), 1.62 (3H, s, Me-18), 3.45 (1H, br m, H-3), 3.88 (1H, q, J=6.5 Hz, H-20), 4.70 (1H, dd, J=10 and 4 Hz, H-12), 5.36 (1H, m, H-6), 6.67 (1H, d, J=16.0 Hz, H-3′), 7.42 (2H, dd, J=7.5 and 1.5 Hz, H-5′ and H-9′), 7.63 (3H, t, J=7.5 Hz, H-6′, H-7′, and H-8′), 7.81 (1H, d, J=16 Hz, H-2′); for the sugar moieties, see Table 2.

3-O-β-D-Oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-12-O-benzoyl-20-O-cinnamoylsarcostin [**9**].—$[\alpha]^{25}D$ +88° (c=1, MeOH); fabms, see text; ^1H nmr δ 1.10 (3H, s, Me-19), 1.34 (3H, d, J=6.6 Hz, Me-21), 1.69 (3H, s, Me-18), 3.50 (1H, br m, H-3), 4.80 (1H, dd, J=10.5 and 4.5 Hz, H-12), 4.85 (1H, q, J=6.6 Hz, H-20), 5.36 (1H, m, H-6), 6.12 (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 and 1.5 Hz, H-5″ and H-9″), 7.50 (1H, d, J=15.9 Hz, H-3″), 7.56 (1H, t, J=7.4 Hz, H-5′), 7.60 (1H, t, J=7.6 Hz, H-6″, H-7″, and H-8″), 8.01 (2H, dd, J=7.4 and 1.2 Hz, H-3′ and H-7′); for the sugar moieties, see Table 2.

BIOLOGICAL ASSAYS.—Raji cells (a human lymphoblastoid cell line from Burkitt's lymphoma) were grown in suspension in RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained in logarithmic growth in a humidified incubator at 37° 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-bottomed 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 (41 Ci/mmol). After 6 h of 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 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). The compounds were tested at doses of 0.5 and 1 μg/ml.

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