Antiproliferative Triterpene Saponins from *Entada africana*[⊥]

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Nine new ester saponins (**1**−**9**) were isolated from the roots of *Entada africana*. Their structures were elucidated by 1D and 2D NMR experiments including 1D and 2D TOCSY, DQF-COSY, HSQC, and HMBC spectroscopy, as well as ESIMS analysis, and chemical methods. The aglycon moieties were found to be echinocystic acid for compounds **1**, **2**, **4**−**6**, **8**, and **9** and acacic acid for **3** and **7**. All isolated compounds were tested for their antiproliferative activity against the J774.A1, HEK-293, and WEHI-164 cell lines. Moderate to high cytotoxic potency was found for almost all compounds tested.

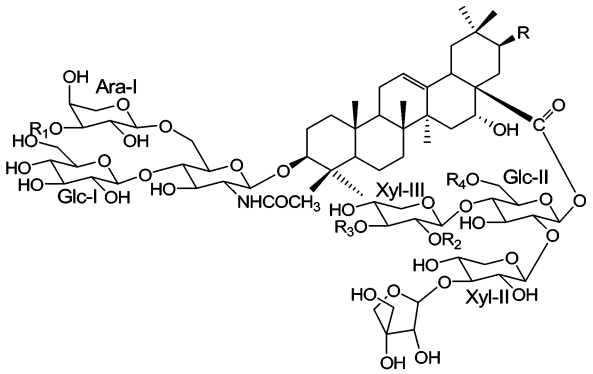
As a part of our investigation on Malian medicinal plants, we have conducted a phytochemical investigation on the roots of *Entada africana* Guill. and Perr. (Leguminosae), local name "Samanèrè", a small tree growing in tropical areas of Africa. The plant is used in traditional medicine for various types of illnesses, and all parts of the tree are used. In Mali, various uses have been made of the anti-inflammatory, hepatoprotective, and wound-healing effects of *E. africana*. Decotions of the root or the bark are used to treat hepatitis and to wash wounds. The juice of the fresh root or the bark is also used for its hemostatic properties. Many other types of uses of *E. africana* are also reported in the literature in different parts of Africa.[1−3]

Previous pharmacological studies on *E. africana* have shown anti-inflammatory, antiulcer, and wound-healing activities.[4−6] At the Departement de Medicine Traditionelle of Bamako, a traditional medicine, "Samanèrè", is used to treat hepatitis. The clinical use of the "Improved Traditional Prescription", which is prepared with the roots of *E. africana*, suggested its effectiveness in hepatoprotection.[7] The antihepatotoxic properties of *E. africana* extracts were evaluated on CCl_4-induced acute liver damage in rats. The results of this study showed that *E. Africana* does afford a protective action against CCl_4-induced hepatocellular injury.[8] There is no report on the secondary metabolites of this plant to date. Previous phytochemical studies of other *Entada* species have reasulted in the occurrence of oleanolic acid, echinocystic acid, entagenic acid, and acacic acid glycosides. [9−11]

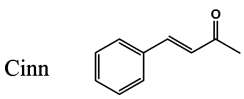
In the present investigation on *E. africana* roots, we report the isolation and structural characterization of nine new triterpenoid ester saponins (**1**−**9**), having echinocystic acid and acacic acid as aglycons. Taking into account the fact that some triterpene saponins have been found to possess cytotoxic activity against various tumor cell lines,[12] all isolated compounds were tested for their antiproliferative activity against the J774.A1, HEK-293, and WEHI-164 cell lines, and the results are reported herein.

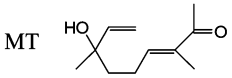
Results and Discussion

The methanol and chloroform−methanol extracts of roots of *E. africana* were subjected separately to Sephadex LH-20 column chromatography, followed by droplet countercurrent chromatography (DCCC), and then RP-HPLC, to afford nine triterpenoid saponins (compounds **1**−**9**).



	R	R_1	R_2	R_3	R_4
1	H	Xyl-I	Cinn	MT	Ac
2	H	Xyl-I	H	MT	Ac
3	OH	Xyl-I	H	MT	Ac
4	H	Xyl-I	Cinn	H	Ac
5	H	Xyl-I	H	H	Ac
6	H	Xyl-I	H	H	H
7	OH	Xyl-I	H	H	Ac
8	H	Ara-II	Cinn	H	Ac
9	H	Ara-II	H	H	Ac





Compound **1** was assigned a molecular formula of $C_{96}H_{143}NO_{43}$, as determined by ^{13}C, ^{13}C DEPT NMR, and ESIMS. The HR/ESIMS of **1** showed a signal at *m*/*z* 2020.9006, consistent with a $[M + Na]^+$ ion (theoretical *m*/*z*: 2020.8932; *m*/*z* difference 5 ppm). In the MS^2 spectrum, prominent fragments at *m*/*z* 1890 $[M + Na - 130]^+$, 1758 $[M + Na - (130 + 132)]^+$, and 1592 $[M + Na - (130 + 132 + 166)]^+$ were observed, due to consecutive losses of one cinnamoyl, one pentose, and one monoterpenoid residue, respectively. However the most abundant species was observed at *m*/*z* 1124 $[M + Na - (130 + 766)]^+$ and was produced by the loss of the whole esterified sugar chain. To obtain structural information on the molecule, a multistage MS^n analysis was performed by fragmentation of the *m*/*z* 1124 parent ion generating a MS^3 spectrum, showing fragments at *m*/*z* 992 $[M + Na - (130 + 766 + 132)]^+$, 962 $[M + Na - (130 + 766 + 162)]^+$, and 860 $[M + Na - (130 + 766 + 132 + 132)]^+$ and suggesting the presence of at least one hexose unit and two pentose residues in the composition of the sugar chain glycosylated at C-3.[13] Finally, a fragment ion was detected at *m*/*z* 652 corresponding to the sodium-cationized

[⊥] Dedicated to the memory of Prof. Arturo Leone, Università di Salerno.

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Table 1. ^{1}H and ^{13}C NMR Data for Sugar Moieties of Compounds **1**, **2**, and **4** (CD_3OD, 600 MHz)[a]

	1		**2**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
3-*O*-sugar						
GlcNAc 1	4.48 d (8.3)	104.8	4.48 d (8.0)	104.8	4.49 d (8.3)	104.5
2	3.73 dd (8.3, 9.8)	57.3	3.73 dd (8.0, 9.8)	57.3	3.73 dd (8.3, 9.8)	57.1
3	3.64 dd (9.8, 10.0)	73.8	3.64 dd (9.8, 10.0)	73.8	3.64 dd (9.8, 10.0)	73.9
4	4.05 dd (10.0, 10.0)	81.4	4.05 dd (10.0, 10.0)	80.5	4.06 dd (10.0, 10.0)	80.5
5	3.52 m	73.9	3.52 m	73.9	3.52 m	75.6
6a	3.96 dd (3.0, 11.5)	68.0	3.96 dd (3.0, 11.5)	68.1	3.96 dd (3.0, 11.5)	68.1
6b	4.12 dd (6.4, 11.5)		4.12 dd (6.4, 11.5)		4.12 dd (6.4, 11.5)	
$-NHCOCH_3$	2.05 s	21.7	2.06 s	21.4	2.02 s	21.4
$-NHCOCH_3$		172.0		172.4		172.5
Glc-I 1	4.65 d (7.8)	104.2	4.65 d (7.8)	104.2	4.64 d (7.8)	104.0
2	3.21 dd (7.8, 9.0)	74.7	3.21 dd (7.8, 9.0)	74.7	3.21 dd (7.8, 9.0)	74.9
3	3.39 dd (9.0, 9.0)	77.9	3.39 dd (9.0, 9.0)	77.9	3.39 dd (9.0, 9.0)	77.8
4	3.37 dd (9.0, 9.0)	71.3	3.37 dd (9.0, 9.0)	71.3	3.37 dd (9.0, 9.0)	71.3
5	3.44 m	77.5	3.44 m	77.7	3.44 m	77.5
6a	3.72 dd (12.0, 3.0)	62.1	3.72 dd (12.0, 3.0)	62.1	3.73 dd (12.0, 3.0)	62.3
6b	3.86 dd (12.0, 5.0)		3.86 dd (12.0, 5.0)		3.88 dd (12.0, 5.0)	
Ara-I 1	4.60 d (7.0)	103.6	4.59 d (6.8)	103.6	4.58 d (7.0)	103.5
2	3.74 dd (8.5, 7.0)	73.9	3.74 dd (8.5, 6.8)	73.9	3.75 dd (8.5, 7.0)	73.9
3	3.77 dd (8.5, 3.0)	83.3	3.77 dd (8.5, 2.5)	83.3	3.77 dd (8.5, 3.0)	83.2
4	3.86 m	69.3	3.86 m	69.3	3.86 m	69.2
5a	3.59 dd (12.0, 3.5)	66.4	3.59 dd (12.0, 3.5)	66.4	3.54 dd (12.0, 3.5)	66.6
5b	4.09 dd (12.0, 2.5)		4.02 dd (12.0, 2.5)		4.09 dd (12.0, 2.5)	
Xyl-I 1	4.50 d (8.0)	107.5	4.50 d (8.0)	107.6	4.50 d (8.0)	107.5
2	3.27 dd (9.5, 8.0)	76.4	3.28 dd (9.5, 8.0)	76.2	3.27 dd (9.5, 8.0)	76.3
3	3.37 dd (9.5, 9.5)	77.9	3.37 dd (9.5, 9.5)	77.9	3.36 dd (9.5, 9.5)	77.8
4	3.51 m	70.0	3.51 m	69.0	3.51 m	69.9
5a	3.25 dd (12.0, 2.0)	67.7	3.27 dd (12.0, 2.0)	67.5	3.25 dd (12.0, 2.0)	67.2
5b	4.06 dd (12.0, 5.0)		4.09 dd (12.0, 5.0)		4.06 dd (12.0, 5.0)	
28-*O*-sugar						
Glc-II 1	5.47 d (7.8)	93.1	5.43 d (7.5)	93.1	5.46 d (7.8)	93.5
2	3.70 dd (7.8, 9.0)	78.7	3.70 dd (7.5, 9.0)	78.7	3.70 dd (7.8, 9.0)	78.7
3	3.83 dd (9.0, 9.0)	76.5	3.83 dd (9.0, 9.0)	76.5	3.83 dd (9.0, 9.0)	76.6
4	3.55 dd (9.0, 9.0)	80.9	3.55 dd (9.0, 9.0)	81.0	3.55 dd (9.0, 9.0)	81.0
5	3.60 m	76.1	3.60 m	76.1	3.60 m	76.0
6a	4.21 dd (12.0, 4.5)	63.1	4.21 dd (12.0, 4.5)	63.1	4.20 dd (12.0, 4.5)	63.6
6b	4.38 dd (12.0, 2.0)		4.28 dd (12.0, 2.0)		4.31 dd (12.0, 2.0)	
$-COCH_3$	2.00 s	20.9	1.99 s	21.0	2.02 s	21.2
$-COCH_3$		172.5		172.6		172.5
Xyl -II 1	4.73 d (7.5)	104.7	4.74 d (7.5)	104.7	4.72 d (7.5)	105.0
2	3.36 dd (9.0, 7.5)	75.3	3.38 dd (9.0, 7.5)	75.3	3.30 dd (9.0, 7.5)	76.3
3	3.41 dd (9.0, 9.0)	85.5	3.42 dd (9.0, 9.0)	85.4	3.40 dd (9.0, 9.0)	85.4
4	3.52 m	71.0	3.54 m	71.0	3.51 m	70.0
5a	3.19 dd (11.0, 5.0)	66.9	3.21 dd (11.0, 5.0)	66.9	3.19 dd (11.0, 5.0)	66.6
5b	3.89 dd (11.0, 2.5)		3.90 dd (11.0, 2.5)		3.92 dd (11.0, 2.5)	
Api 1	5.29 d (3.0)	111.1	5.28 d (2.8)	111.1	5.29 d (3.0)	111.5
2	4.00 br s	77.9	3.99 br s	77.9	4.00 br s	77.9
3		80.5		80.4		80.4
4a	3.83 d (10.0)	75.2	3.83 d (9.8)	75.0	3.96 d (9.8)	75.3
4b	4.16 d (10.0)		4.16 d (9.8)	65.2	4.15 d (9.8)	
5	3.64 br s	65.2	3.64 br s		3.64 br s	65.2
Xyl-III 1	4.58 d (7.8)	104.6	4.58 d (8.0)	104.6	4.57 d (7.8)	103.5
2	4.83 dd (9.0, 7.8)	75.0	3.38 dd (9.0, 8.0)	75.3	4.87 dd (9.0, 7.8)	75.0
3	4.70 dd (9.0, 9.0)	79.0	4.70 dd (9.0, 9.0)	79.0	3.32 dd (9.0, 9.0)	75.3
4	3.64 m	71.0	3.60 m	70.4	3.50 m	69.9
5a	3.37 dd (11.0, 5.0)	67.3	3.27 dd (11.0, 5.0)	67.3	3.36 dd (11.0, 5.0)	67.3
5b	3.89 dd (11.0, 2.5)		3.89 dd (11.0, 2.5)		3.86 dd (11.0, 2.5)	

[a] *J* values are in parentheses and reported in Hz; assignments were confirmed by DQF-COSY, 1D TOCSY, HSQC, and HMBC experiments.

etherified sugar portion of compound **1**, followed by fragment peaks at *m*/*z* 520, 388, and 490, generated by the elimination of one or two pentose units or one hexose residue, respectively. Accordingly, compound **1** was determined as having a triterpene skeleton bearing one esterified and one etherified sugar chain of molecular weight 896 and 629, respectively. In order to identify the esterified substituents present in the molecule, compound **1** underwent alkaline hydrolysis. LC-MS analysis of the reaction mixture revealed the presence of three main ions at *m*/*z* 1868, 1832, and 1102, corresponding to the protonated fragments generated by the elimination of one cinnamoyl unit, one monoterpenoid unit, or the whole esterified saccharidic moiety from compound **1**, respectively.

The ^{13}C and ^{13}C DEPT spectra (Table 1 and Experimental Section) of **1** showed 96 signals, of which 30 were assigned to the aglycon, 43 to the sugar portion, and 23 to acyl groups. Data from the ^{13}C NMR spectrum (see Experimental Section and Table 1) suggested a triterpenoid glycoside structure. The ^{1}H NMR spectrum of the aglycon portion of **1** showed signals for seven tertiary methyl groups (δ 0.80, 0.82, 0.86, 0.95, 1.00, 1.02, 1.38), a typical signal of H-3ax at δ 3.14 (dd, J = 11.5, 4.0 Hz), a signal of H-16eq at δ 4.56 (br m), and a characteristic olefinic proton at δ 5.32 (t, J = 2.5 Hz). The ^{13}C NMR spectrum showed for the aglycon moiety signals that could be correlated unambiguously to the corresponding proton chemical shifts from the HSQC experiment, leading to the

identification of the aglycon as echinocystic acid.[14] However, the ^{1}H NMR data displayed signals for two tertiary methyls (δ 1.36 and 1.86), three coupled olefinic protons (δ 5.93, 5.17, and 5.08), and one olefinic proton (δ 6.85), suggesting the presence of a monoterpene (MT) unit. The NMR signal assignments of the MT unit were obtained by comparison with NMR data of similar structures in the literature and subsequently confirmed by analysis of 2D NMR spectra. Alkaline hydrolysis with $NaHCO_3-H_2O$ of compound **1** gave this MT moiety. The absolute configuration for C-6 of the MT moiety was determined to be 6*R* by the rotation of MT, $[\alpha]_D^{25} = -15.5$, in a direct comparison with (6*R*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid. Thus, the MT moiety was identified as (2*E*,6*R*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid.[15,16] In the ^{1}H NMR spectrum of **1** were also identified signals for two coupled doublets of *trans*-olefinic protons (δ 7.92, 6.78) and five aromatic protons characteristic of a monosubstituted phenyl group for a cinnamoyl moiety.

The sugar portion of **1** exhibited, in the ^{1}H NMR spectrum, eight anomeric proton signals (δ 4.48, d, $J = 8.3$ Hz; 4.50, d, $J = 8.0$ Hz; 4.58, d, $J = 7.8$ Hz; 4.60, d, $J = 7.0$ Hz; 4.65, d, $J = 7.8$ Hz; 4.73, d, $J = 7.5$ Hz; 5.47, d, $J = 7.8$ Hz; 5.29, br d, $J = 2.0$ Hz). The structures of the oligosaccharide moieties were deduced using ESIMS, 1D TOCSY, and 2D NMR experiments, which indicated that two β-glucopyranoses, three β-xylopyranoses, one α-arabinopyranose, and one β-apiofuranose were present (Table 1). The eighth sugar was determined as an *N*-acetylated aminoglucopyranosyl unit. The presence of an acetamido group was suggested by the detection of signals at δ_H 2.05 (3H, s), δ_C 21.7, and δ_C 172.0 ppm as well as by the correlation observed in an HMBC experiment between $-$CONH (δ_C 172.0) and H-2 (d_H 3.73) of the glucopyranosyl ring. The shielding of C-2 to δ_C 57.1 is characteristic of a 1-deoxy-2-acetoamidoglucosyl moiety.[17] The HSQC, COSY, and 1D TOCSY spectra of **1** also showed acylation shifts for H-6 of glucopyranosyl-II (δ_H 4.21, and 4.38) and C-6 (δ_C 63.1) and for one xylopyranosyl residue H-2 (δ_H 4.83) and C-2 (δ_C 75.0); H-3 (δ_H 4.70) and C-3 (δ_C 79.0). The absolute configurations of the sugar units were assigned after hydrolysis of all compounds with 1 N HCl. The hydrolyzate was trimethylsilylated, and GC retention times of each sugar were compared with those of authentic sugar samples prepared in the same manner. The apiofuranosyl ring configuration was also confirmed by comparing ^{1}H$-^1$H scalar coupling constants with those reported for methyl apiofuranosides and DL-apioses and by NOE observations.[18] The 2D NOESY spectrum of **1** showed cross-peaks between H-2 and the protons of the hydroxymethyl group, and H-2 and H-4b, indicating that H-2, the hydroxymethyl group, and H-4b are found on the same face of the ring for this sugar and confirming its structure as β-D-apiose. The absence of any ^{13}C NMR glycosidation shift for one β-D-glucopyranosyl, two β-D-xylopyranosyls, and the β-D-apiofuranosyl moiety suggested that these sugars are terminal units. Glycosidation shifts were observed for C-6_{glcNAc} (68.0 ppm), C-4_{glcNAc} (81.4 ppm), C-3_{ara} (83.2 ppm), C-2_{glcII} (78.9 ppm), C-4_{glcII} (80.1 ppm), and C-3_{xylII} (85.5 ppm) (Table 1). The chemical shifts of H-1_{glcII} (δ 5.47) and C-1_{glcII} (93.1 ppm) indicated that this sugar unit is involved in an ester linkage with the C-28 carboxylic group.[19] Direct evidence for the sugar sequence linked at C-3 was derived from the results of the HMBC experiment, which showed unequivocal correlation peaks between resonances at δ_H 4.48 and 91.0 ppm (H-$1_{glcNAc}-$C-3), δ_H 4.65 and 81.4 ppm (H-$1_{glcI}-$C-4_{glcNAc}), δ_H 4.50 and 83.3 ppm (H-$1_{xylI}-$C-3_{araI}), and δ_H 4.60 and 68.0 ppm (H-$1_{araI}-$C-6 $_{glcNAc}$). Similarly, the sequence of the tetrasaccharide chain at C-28 was indicated by the cross-peaks between δ_H 4.73 and 78.9 ppm (H-$1_{xylII}-$C-2_{glcII}), δ_H 5.29 and 85.5 ppm (H-$1_{api}-$C-3_{xylII}), and δ_H 4.58 and 80.1 ppm (H-$1_{xylIII}-$C-4_{glcII}).

It only remained to establish the relative positions of the cinnamoyl, monoterpenoid, and acetyl residues at C-2, C-3 of xyl_{III} and C-6 of glc_{II} in the structure of **1**. To confirm the position of the ester linkage, a HMBC experiment was carried out. Since the carbonyl carbon of the monoterpenoyl moiety (δ_C 169.0 ppm) showed a correlation with H-3_{xylIII} (δ_H 4.70 ppm), and the carbonyl carbon of the cinnamoyl moiety (δ_C 167.5 ppm) correlated with H-2_{xylIII} (δ_H 4.83 ppm), it was concluded that a cinnamoyl residue esterified the hydroxyl group at C-2 and the monoterpene moiety occurred at C-3 of xyl_{III}. Consequently, the acetyl group was located at C-6 of glc_{II}. On the basis of all of this evidence, compound **1** was assigned as 3β-*O*-{β-D-xylopyranosyl-(1$\rightarrow$3)-α-L-arabinopyranosyl-(1$\rightarrow$6)-[β-D-glucopyranosyl-(14)]-2-(acetamido)-2-deoxy-β-D-glucopyranosyl}echinocystic acid 28-*O*-{β-D-apiofuranosyl-(1$\rightarrow$3)-β-D-xylopyranosyl-(1$\rightarrow$2)-[(2-*O*-cynnamoyl), (3-*O*-(2*E*,6*R*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-xylopyranosyl-(1$\rightarrow$4)]-(6-*O*-acetyl)-β-D-glucopyranosyl ester.

Compound **2** showed a molecular formula of $C_{87}H_{137}NO_{42}$, established unequivocally from the HR/ESIMS (sodiated molecular ion peak at *m*/*z* 1890.8705 $[M + Na]^+$, theoretical value 1890.8513) and its NMR data and by elemental analysis. MS^n spectra obtained for this compound showed the same fragments detected in the case of compound **1** starting from the fragment at *m*/*z* 1891. The ^{1}H and ^{13}C NMR chemical shifts (Table 1) of compounds **2** and **1** were almost superimposable, with the absence of the cinnamoyl group linked at C-2 of the xyl_{III} being the major difference between these compounds (H-2_{xylIII} at δ 4.83 in **1** versus H-2_{xylIII} δ 3.38 in **2**). Thus, the structure of compound **2** was established as 3β-*O*-{β-D-xylopyranosyl-(1$\rightarrow$3)-α-L-arabinopyranosyl-(1$\rightarrow$6)-[β-D-glucopyranosyl-(1$\rightarrow$4)]-2-(acetamido)-2-deoxy-β-D-glucopyranosyl}-echinocystic acid 28-*O*-{β-D-apiofuranosyl-(1$\rightarrow$3)-β-D-xylopyranosyl-(1$\rightarrow$2)-[(3-*O*-(2*E*,6*R*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-xylopyranosyl-(1$\rightarrow$4)]-(6-*O*-acetyl)-β-D-glucopyranosyl ester.

Compound **3** was assigned the molecular formula $C_{87}H_{137}NO_{43}$. The HR/ESIMS showed the $[M + Na]^+$ ion at *m*/*z* 1906.8583 (theoretical value 1906.8462). In the MS^2 spectrum, fragments at *m*/*z* 1722 $[M + Na - 184]^+$ and 1642 $[M + Na - 184 - 132]^+$ were observed, due to subsequent losses of a monoterpenoid unit and a pentose unit. Analysis of the NMR data of compound **3** (Experimental Section) and comparison with those of **2** showed that they possess the same saccharide chains at C-3 and C-28, while the compounds are based on different aglycons. The main differences were the downfield shift of C-21 (δ_C 77.6) and C-22 (δ_C 36.5) in the ^{13}C NMR spectrum and the downfield shift of the methyl at C-30 (δ_H 1.15) in the ^{1}H NMR spectrum, implying an additional hydroxyl group at C-21 of the aglycon of **3**. This hypothesis was in agreement with the MS data and was confirmed unambiguously by the HMBC spectrum, which showed cross-peaks between the proton at δ_H 5.08 and the C-19, C-17, and C-30 signals. The β-configuration of the C-21 hydroxyl group was evident from the chemical shift and the large *J* value of H-21 (1H, dd, $J = 11.2$, 5.5 Hz), characteristic of an axial proton. Thus, the aglycon of **3** was identified as acacic acid.[20,21] Therefore, compound **3** was defined as 3β-*O*-{β-D-xylopyranosyl-(1$\rightarrow$3)-α-L-arabinopyranosyl-(1$\rightarrow$6)-[β-D-glucopyranosyl-(1$\rightarrow$4)]-2-(acetamido)-2-deoxy-β-D-glucopyranosyl}acacic acid 28-*O*-{β-D-apiofuranosyl-(1$\rightarrow$3)-β-D-xylopyranosyl-(1$\rightarrow$2)-[(3-*O*-(2*E*,6*R*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-xylopyranosyl-(1$\rightarrow$4)]-(6-*O*-acetyl)-β-D-glucopyranosyl ester.

Compound **4** was assigned the molecular formula $C_{86}H_{129}NO_{41}$, as established unequivocally by the ESIMS (pseudomolecular ion peak at *m*/*z* 1854 $[M + Na]^+$) and from its NMR and elemental analysis data. In the MS^2 spectrum, fragment ions at *m*/*z* 1724 $[M + Na - 130]^+$ and 1812 $[M + Na - 42]^+$ were observed, indicating the loss of one cinnamoyl unit or of one acetyl unit; the MS^3 spectrum showed a largely predominant fragment at *m*/*z* 1124 $[M + Na - (130 + 766)]^+$, produced by the loss of an esterified sugar chain. Also in this case, a fragment ion at *m*/*z* 652 corresponding to the sodium-cationized etherified sugar portion was detected. The ^{1}H and ^{13}C NMR chemical shifts (Table 1) of compounds **4** and **1**

were almost superimposable, with the absence of the monoterpenoyl group linked at C-3 of the xyl_{III} unit being the point of difference for compound **4** (H-3_{xylIII} at δ 4.70 in **1** versus H-3_{xylIII} at δ 3.32 in **4**). Thus, the structure of compound **4** was established as 3*β*-*O*-{*β*-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}-echinocystic acid 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-[(2-*O*-cinnamoyl)-*β*-D-xylopyranosyl-(1→4)]-(6-*O*-acetyl)-*β*-D-glucopyranosyl ester.

The ESIMS of compound **5** ($C_{77}H_{123}NO_{40}$) showed a single ion at *m*/*z* 1724 ($[M + Na]^+$) and a fragmentation pattern similar to that of **4**. The spectroscopic data of the aglycon moiety of **5** were identical to those of **4**. Comparison of the NMR data of the sugar moieties (Table 2 and Experimental Section) of **5** with those of **4** indicated that **5** differs from **4** only by the absence of the cinnamoyl group linked at C-2 of the xyl_{III} unit. Thus, compound **5** was determined as 3*β*-*O*-{*β*-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}echinocystic acid 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-[(-*β*-D-xylopyranosyl-(1→4)]-(6-*O*-acetyl)-*β*-D-glucopyranosyl ester.

The ESIMS of compound **6** ($C_{75}H_{121}NO_{39}$) showed a main signal at *m*/*z* 1682 ($[M + Na]^+$), and MS^n analyses gave results similar to those obtained for compound **5**. The spectroscopic data of the aglycon moiety of **6** were identical to those of **5**. Comparison of NMR spectroscopic data of the sugar moieties of **6** with those of **5** indicated that **6** differed from **5** only by the absence of the acetyl group linked at C-6 of the glc_{II} unit (Table 2). Thus, compound **6** was determined as 3*β*-*O*-{*β*-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}echinocystic acid 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-[(*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranosyl ester.

Compound **7** ($C_{77}H_{123}NO_{41}$) exhibited an ESIMS showing the $[M + Na]^+$ peak at *m*/*z* 1740 and a fragmentation pattern similar to that of **3**. The NMR aglycon signals were identical to those of **3**, allowing the aglycon of **7** to be identified as acacic acid. Analysis of the NMR data of the sugar chains of compound **7** demonstrated that they were superimposable with those of **5**. Thus, the structure 3*β*-*O*-{*β*-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}acacic acid 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-[(-*β*-D-xylopyranosyl-(1→4)]-(6-*O*-acetyl)-*β*-D-glucopyranosyl ester was assigned to compound **7**.

Compound **8** (molecular formula $C_{86}H_{129}NO_{41}$) showed a quasi-molecular ion peak at *m*/*z* 1854 $[M + Na]^+$ in the positive ESIMS. Furthermore, in the MS^2 spectrum a fragment ion peak at *m*/*z* 1724 $[M + Na - 130]^+$ was detected, indicating the loss of one cinnamoyl unit. The ^{13}C NMR spectrum (Table 3 and Experimental Section) showed 86 signals, of which 30 were assigned to a triterpenoid moiety, 43 to the saccharide portion, and 13 to the acetyl and cinnamoyl residues. Analysis of the NMR data (Table 3) of compound **8** and comparison with those of **4** revealed **8** to differ from **4** only in the sugar chain at C-3. The proton coupling network within each sugar residue was traced out, using a combination of 1D TOCSY, DQF-COSY, and HSQC NMR experiments. Once again, direct evidence for the sugar sequence and the linkage sites was derived from the HSQC and HMBC data. These results established that the tetrasaccharide chain at C-3 of **8** contains a terminal *β*-glucopyranose, a terminal α-arabinopyranose, an inner α-arabinopyranose substituted at C-3, and an inner 2-(acetylamino)-2-deoxyglucopyranosyl disubstituted at C-4 and C-6. On the basis of these data, the structure of **8** was determined as 3*β*-*O*-{α-L-arabinopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}echinocystic acid 28-*O*-{*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-[(2-*O*-cinnamoyl)-*β*-D-xylopyranosyl-(1→4)]-(6-*O*-acetyl)-*β*-D-glucopyranosyl ester.

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

	5		**6**	
	δ_H	δ_C	δ_H	δ_C
3-*O*-sugar				
GlcNAc 1	4.49 d (8.3)	104.8	4.48 d (8.0)	105.2
2	3.73 dd (8.3, 9.8)	57.1	3.74 dd (8.0, 9.8)	57.1
3	3.64 dd (9.8, 10.0)	73.9	3.64 dd (9.8, 10.0)	74.0
4	4.05 dd (10.0, 10.0)	81.4	4.06 dd (10.0, 10.0)	80.6
5	3.52 m	75.0	3.52 m	75.1
6a	3.96 dd (3.0, 11.5)	68.0	3.95 dd (3.0, 11.5)	68.1
6b	4.12 dd (6.4, 11.5)		4.15 dd (6.4, 11.5)	
$-NHCOCH_3$	2.05 s	21.7	2.04 s	21.4
$-NHCOCH_3$		172.0		172.4
Glc-I 1	4.62 d (7.8)	104.0	4.65 d (7.8)	104.3
2	3.21 dd (7.8, 9.0)	74.7	3.22 dd (7.8, 9.0)	74.7
3	3.39 dd (9.0, 9.0)	77.8	3.39 dd (9.0, 9.0)	77.8
4	3.37 dd (9.0, 9.0)	71.3	3.37 dd (9.0, 9.0)	71.1
5	3.44 m	77.5	3.46 m	77.6
6a	3.72 dd (12.0, 3.0)	62.1	3.72 dd (12.0, 3.0)	62.9
6b	3.87 dd (12.0, 5.0)		3.89 dd (12.0, 5.0)	
Ara-I 1	4.56 d (7.0)	103.5	4.57 d (6.8)	103.7
2	3.73 dd (8.5, 7.0)	73.9	3.74 dd (8.5, 6.8)	73.9
3	3.71 dd (8.5, 3.0)	83.2	3.76 dd (8.5, 2.5)	83.3
4	3.86 m	69.3	3.87 m	69.0
5a	3.57 dd (12.0, 3.5)	67.2	3.55 dd (12.0, 3.5)	66.3
5b	4.09 dd (12.0, 2.5)		3.88 dd (12.0, 2.5)	
Xyl-I 1	4.49 d (8.0)	107.3	4.48 d (8.0)	107.6
2	3.27 dd (9.5, 8.0)	76.3	3.28 dd (9.5, 8.0)	76.4
3	3.36 dd (9.5, 9.5)	77.8	3.34 dd (9.5, 9.5)	77.8
4	3.51 m	68.9	3.53 m	69.9
5a	3.23 dd (12.0, 2.0)	66.5	3.27 dd (12.0, 2.0)	66.5
5b	3.96 dd (12.0, 5.0)		3.87 dd (12.0, 5.0)	
28-*O*-sugar				
Glc-II 1	5.47 d (7.8)	93.5	5.48 d (7.5)	93.6
2	3.70 dd (7.8, 9.0)	78.9	3.69 dd (7.5, 9.0)	78.8
3	3.80 dd (9.0, 9.0)	76.6	3.82 dd (9.0, 9.0)	76.7
4	3.58 dd (9.0, 9.0)	80.1	3.64 dd (9.0, 9.0)	79.9
5	3.72 m	74.5	3.50 m	77.0
6a	4.41 dd (12.0, 4.5)	63.8	3.80 dd (12.0, 4.5)	61.0
6b	4.32 dd (12.0, 2.0)		3.86 dd (12.0, 2.0)	
$-COCH_3$	2.00 s	20.9		
$-COCH_3$		172.5		
Xyl-II 1	4.73 d (7.5)	104.7	4.72 d (7.5)	104.7
2	3.30 dd (9.0, 7.5)	75.3	3.34 dd (9.0, 7.5)	75.3
3	3.40 dd (9.0, 9.0)	85.5	3.41 dd (9.0, 9.0)	85.9
4	3.51 m	70.2	3.52 m	70.9
5a	3.20 dd (11.0, 5.0)	66.8	3.18 dd (11.0, 5.0)	66.4
5b	3.92 dd (11.0, 2.5)		3.97 dd (11.0, 2.5)	
Api 1	5.29 d (2.8)	111.2	5.28 d (2.8)	111.3
2	3.99 br s	77.9	3.99 br s	77.9
3		80.6		80.7
4a	3.81 d (9.8)	75.1	3.83 d (9.8)	74.9
4b	4.16 d (9.8)		4.13 d (9.8)	65.1
5	3.66 br s	65.2	3.63 br s	
Xyl-III 1	4.27 d (7.8)	105.2	4.37 d (8.0)	105.3
2	3.21 dd (9.0, 7.8)	74.3	3.21 dd (9.0, 8.0)	74.7
3	3.31 dd (9.0, 9.0)	77.8	3.31 dd (9.0, 9.0)	77.8
4	3.40 m	70.0	3.48 m	70.9
5a	3.20 dd (11.0, 5.0)	66.5	3.20 dd (11.0, 5.0)	66.4
5b	3.89 dd (11.0, 2.5)		3.89 dd (11.0, 2.5)	

[a] *J* values are in parentheses and reported in Hz; assignments were confirmed by DQF-COSY, 1D TOCSY, HSQC, and HMBC experiments.

The ESIMS of compound **9** ($C_{77}H_{123}NO_{40}$) showed a main signal at *m*/*z* 1724 ($[M + Na]^+$) and a fragmentation pattern similar to that of **8**. Comparison of the NMR spectroscopic data of the sugar moieties (Table 3 and Experimental Section) of **9** with those of **8** indicated that **9** differs from **8** only by the absence of the cinnamoyl group linked at C-2 of the xyl_{III} unit. Thus, compound **9** was determined as 3*β*-*O*-{α-L-arabinopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(14)]-2-(acetamido)-2-deoxy-*β*-D-glucopyranosyl}echinocystic acid 28-*O*-{*β*-d-apiofuranosyl-(1→3)-

Table 3. ^{1}H and ^{13}C NMR Data for Sugar Moieties of Compounds **8** and **9** (CD_3OD, 600 MHz)[a]

	8		**9**	
	δ_H	δ_C	δ_H	δ_C
3-*O*-sugar				
GlcNAc 1	4.48 d (8.3)	104.6	4.46 d (7.6)	104.7
2	3.72 dd (8.3, 9.8)	57.0	3.72 dd (7.6, 9.8)	57.1
3	3.64 dd (9.8, 10.0)	74.0	3.63 dd (10.0, 10.0)	74.0
4	4.05 dd (10.0, 10.0)	80.7	4.04 dd (10.0, 10.0)	80.8
5	3.52 m	73.9	3.49 m	75.0
6a	3.92 dd (3.0, 11.5)	67.9	3.96 dd (3.0, 11.5)	68.0
6b	4.12 dd (6.4, 11.5)		4.14 dd (6.4, 11.5)	
$-NHCOCH_3$	2.04 s	21.3	2.00 s	21.1
$-NHCOCH_3$		172.4		172.3
Glc-I 1	4.64 d (7.8)	104.2	4.63 d (7.8)	104.2
2	3.22 dd (7.8, 9.0)	74.7	3.25 dd (7.8, 9.0)	74.7
3	3.40 dd (9.0, 9.0)	77.9	3.36 dd (9.0, 9.0)	77.8
4	3.37 dd (9.0, 9.0)	71.3	3.39 dd (9.0, 9.0)	71.2
5	3.45 m	77.7	3.44 m	77.6
6a	3.74 dd (12.0, 3.0)	62.1	3.74 dd (12.0, 3.0)	62.2
6b	3.88 dd (12.0, 5.0)		3.88 dd (12.0, 5.0)	
Ara -I 1	4.60 d (7.0)	103.6	4.61 d (6.8)	103.7
2	3.72 dd (8.5, 7.0)	73.9	3.74 dd (8.5, 6.8)	73.9
3	3.78 dd (8.5, 3.0)	84.0	3.79 dd (8.5, 2.5)	84.1
4	3.83 m	69.1	3.84 m	69.0
5a	3.59 dd (12.0, 3.5)	66.5	3.57 dd (12.0, 3.5)	66.5
5b	4.02 dd (12.0, 2.5)		4.00 dd (12.0, 2.5)	
Ara-II 1	4.58 d (7.0)	103.8	4.58 d (8.0)	103.9
2	3.68 dd (8.5, 7.0)	74.4	3.69 dd (9.5, 8.0)	74.1
3	3.73 dd (8.5, 3.0)	71.0	3.71 dd (9.5, 2.5)	71.0
4	3.86 m	69.3	3.83 m	69.3
5a	3.56 dd (12.0, 2.3)	66.4	3.57 dd (12.0, 2.0)	66.4
5b	3.89 br d (12.3)		3.89 dd (12.0, 5.0)	
28-*O*-sugar				
Glc-II 1	5.40 d (7.8)	93.1	5.45 d (7.5)	93.3
2	3.71 dd (7.8, 9.0)	78.7	3.70 dd (7.5, 9.0)	78.8
3	3.83 dd (9.0, 9.0)	76.5	3.80 dd (9.0, 9.0)	76.6
4	3.55 dd (9.0, 9.0)	81.0	3.65 dd (9.0, 9.0)	81.0
5	3.60 m	76.1	3.58 m	76.1
6a	4.21 dd (12.0, 4.5)	63.8	4.24 dd (12.0, 4.5)	63.8
6b	4.28 dd (12.0, 2.0)		4.31 dd (12.0, 2.0)	
$-COCH_3$	2.00 s	20.7	1.99	21.0
$-COCH_3$		172.5		172.4
Xyl-II 1	4.74 d (7.5)	104.6	4.72 d (7.5)	104.7
2	3.35 dd (9.0, 7.5)	75.3	3.34 dd (9.0, 7.5)	75.3
3	3.40 dd (9.0, 9.0)	85.9	3.41 dd (9.0, 9.0)	85.9
4	3.52 m	70.8	3.52 m	70.8
5a	3.18 dd (11.0, 5.0)	66.4	3.16 dd (11.0, 5.0)	66.4
5b	3.97 dd (11.0, 2.5)		3.95 dd (11.0, 2.5)	
Api 1	5.29 d (3.0)	111.1	5.28 d (3.0)	111.3
2	4.01 br s	77.9	3.99 br s	77.9
3		80.6		80.7
4a	3.85 d (9.6)	75.2	3.83 d (9.8)	74.9
4b	4.14 d (9.6)		4.13 d (9.8)	65.4
5	3.63 br s	65.4	3.64 br ss	
Xyl-III 1	4.55 d (7.8)	103.5	4.30 d (8.0)	104.0
2	4.87 dd (9.0, 7.8)	75.0	3.21 dd (9.0, 8.0)	74.3
3	3.32 dd (9.0, 9.0)	75.3	3.31 dd (9.0, 9.0)	77.8
4	3.50 m	69.9	3.43 m	70.0
5a	3.36 dd (11.0, 5.0)	67.3	3.20 dd (11.0, 5.0)	66.5
5b	3.86 dd (11.0, 2.5)		3.89 dd (11.0, 2.5)	

[a] *J* values are in parentheses and reported in Hz; assignments were confirmed by DQF-COSY, 1D TOCSY, HSQC, and HMBC experiments.

β-D-xylopyranosyl-(1→2)-[(β-D-xylopyranosyl-(1→4)]-(6-*O*-acetyl)-β-D-glucopyranosyl ester.

Since some triterpene saponins from the family Leguminosae have been reported to be cytotoxic for tumor cell lines,[15,21−24] compounds **1**−**9** were tested in vitro against three cell lines, comprising J774.A1 (murine monocyte/macrophage), HEK-293 (human epithelial kidney), and WEHI-164 (murine fibrosarcoma). Compounds **1**−**3** showed cytotoxic activity against all the cell lines used (Table 4), and among these compounds, compound **3** showed the highest cytotoxic activity. The structures of **1**−**3** are very similar except for the substituent at C-21, and this indicates that the OH group at C-21 might influence the resultant cytotoxic activity. Compounds **4**, **7**, and **8** showed moderate activity, while compounds **5**, **6**, and **9** displayed activity against J774.A1 cells but no activity against the other cell lines. The cytotoxic effects of compounds **1**−**9** were dependent on the substituents present, with those having cinnamic and/or monoterpenoid residues being more potent when compared with those without esterified groups. A possible explanation is that these moieties determine the overall hydrophilic properties of the compounds, and the more hydrophilic compounds are less able to pass through the cell membrane of mammalian cells, which is reflected in a lower cytotoxicity.

Table 4. In Vitro Antiproliferative Activity of Compounds **1**−**9**[a]

	cell line (IC_{50} μM)		
compound	J774.A1[b]	HEK-293[c]	WEHI-164[d]
1	0.84 ± 0.006	0.36 ± 0.004	0.38 ± 0.003
2	0.52 ± 0.003	0.27 ± 0.002	0.49 ± 0.001
3	0.031 ± 0.002	0.25 ± 0.011	0.10 ± 0.002
4	0.98 ± 0.001	0.56 ± 0.012	0.94 ± 0.034
5	2.9 ± 0.012	nd[e]	nd[e]
6	1.2 ± 0.051	nd[e]	nd[e]
7	2.2 ± 0.007	3.9 ± 0.021	2.0 ± 0.023
8	3.0 ± 0.032	2.8 ± 0.004	2.7 ± 0.004
9	3.1 ± 0.022	nd[e]	nd[e]
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 a 50% reduction in cell growth (after a 3-day incubation). [b] J774.A1 = murine monocyte/macrophage cell lines. [c] HEK-293 = human epithelial kidney cell lines. [d] WEHI-164 = murine fibrosarcoma cell lines. [e] nd = not detectable. [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 achieve frequency discrimination in the ω_1 dimension. Standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, and HMBC NMR experiments. NMR data were processed on a Silicon Graphics Indigo2 workstation using UXNMR software. ESIMS (positive mode) were obtained from a LCQ Advantage Thermo Electron spectrometer, equipped with Xcalibur software. HRMS were acquired on a Q-TOF Premier mass spectrometer (Waters) equipped with an electrospray ion source. LC-MS analysis was performed on a Surveyor HPLC system (Thermo Electron Corporation) coupled with an LCQ Advantage electrospray mass spectrometer. Chromatographic separation was conducted on a C_{18} Synergy Fusion (15 cm × 2.1 mm, flow rate 0.2 mL min^{-1}) column (Phenomenex), using a linear gradient from 40% to 70% of 0.5% formic acid and 0.1% TFA−acetonitrile over 30 min. Elemental analysis was obtained from a Carlo Erba 1106 elemental analyzer. Column chromatography was performed over Sephadex LH-20 (Pharmacia). Droplet counter current chromatography (DCCC) was performed on an apparatus manufactured by Buchi, equipped with 300 tubes. HPLC separations were conducted on a Waters 590 system equipped with a Waters R401 refractive index detector and a Waters C_{18} μ-Bondapak column (30 cm × 7.8 mm) using a U6K injector. GC analyses were performed using a Dani GC 1000 instrument on an l-Chirasil-Val column (0.32 mm × 25 m). Analytical TLC was performed on precoated Kieselgel 60 F254 plates (Merck), and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. The roots of *Entada africana* were collected near Bamako, Mali, October 2002 and identified by Mr. Amey Tapily. A voucher specimen (DMT No 0114) has been deposited in the Herbarium of the Departement de Medicine Traditionelle, Bamako, Mali.

Extraction and Isolation. The dried, powdered roots of *E. africana* (180 g) were defatted with *n*-hexane and successively extracted for 48 h with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH, by exhaustive

maceration (3 × 2 L), to give 4.5, 6.0, and 26.0 g of the respective residues. The methanol extract was partitioned between *n*-BuOH and H_2O, to afford a *n*-BuOH-soluble portion (7.0 g). The *n*-BuOH residue (5 g) was submitted to a Sephadex LH-20 column (5 × 100 cm) using MeOH as eluent to obtain eight major fractions (1−8) by analytical TLC on silica 60 F_{254} gel-coated glass sheets with *n*-BuOH−AcOH−H_2O (60:15:25) and $CHCl_3$−MeOH−H_2O (40:9:1). Fractions 2 and 3 (550 mg) were purified by DCCC with $CHCl_3$−MeOH-H_2O−*i*-PrOH (5:6:4:1), in which the stationary phase consisted of the lower phase (ascending mode), with fractions of 5 mL collected. DCCC fractions 160−202 (50 mg) were chromatographed by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL min^{-1}), with MeOH−H_2O (65:35), to afford pure compounds **6** (14.0 mg, t_R = 30 min) and **7** (9.5 mg, t_R = 33 min). DCCC fractions 204−298 (70 mg) and 301−325 (25 mg) were separately purified by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}), with MeOH−H_2O (7:3), to yield pure compounds **6** (4.5 mg, t_R = 24 min) and **5** (20 mg, t_R = 27 min), from fractions 204−298, and compound **9** (6.0 mg, t_R = 29 min) from fractions 301−325, respectively. Fraction 5 (800 mg) was purified by DCCC with *n*-BuOH−Me_2CO−H_2O (30:9:11), in which the stationary phase consisted of the upper phase (descending mode), again with fractions of 5 mL collected. DCCC fractions 80−170 (150 mg) were subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH−H_2O (7:3) to yield pure compounds **3** (9 mg, t_R = 18 min), **8** (15 mg, t_R = 20 min), and **4** (22 mg, t_R = 27 min). DCCC fractions 184−230 (143 mg) and 230−260 (82 mg) were further separated by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}), with MeOH−H_2O (7.5:2.5), to give pure compounds **3** (3.1 mg, t_R = 15 min), **8** (7.8 mg, t_R = 18 min), and **2** (2.6 mg, t_R = 22 min), from fractions 184−230, as well as compounds **2** (12.5 mg, t_R = 22 min) and **1** (35 mg, t_R = 31 min) from fractions 230−260, respectively.

Compound 1: white solid; $[\alpha]_D^{25}$ −51 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon (CD_3OD, 600 MHz) δ 0.80 (3H, s, Me-24), 0.82 (3H, s, Me-26), 0.86 (3H, s, Me-25), 0.95 (3H, s, Me-29), 1.00 (3H, s, Me-23), 1.02 (3H, s, Me-30), 1.38 (3H, s, Me-27), 3.01 (1H, dd, J = 12.8, 4.5 Hz, H-18), 3.14 (1H, dd, J = 11.5, 4.0 Hz, H-3), 4.56 (1H, br m, H-16), 5.32 (1H, t, J = 2.5 Hz, H-12); ^{13}C NMR data of the aglycon (CD_3OD, 150 MHz) δ 16.0 (C-26), 16.6 (C-25), 17.8 (C-24), 18.5 (C-6), 24.5 (C-30), 23.8 (C-11), 26.6 (C-2), 27.7 (C-27), 29.3 (C-23), 30.8 (C-20), 33.0 (C-29), 34.2 (C-7), 33.0 (C-21), 36.0 (C-22), 36.6 (C-15), 37.8 (C-10), 39.0 (C-4), 39.8 (C-1), 40.6 (C-8), 42.0 (C-14), 41.9 (C-18), 47.7 (C-19), 50.0 (C-17), 48.2 (C-9), 57.2 (C-5), 74.6 (C-16), 91.0 (C-3), 123.7 (C-12), 144.0 (C-13), 177.6 (C-28); ^{1}H NMR data of the monoterpenoid moiety (CD_3OD, 600 MHz) δ 1.20 (1H, t, J = 6.6 Hz, H-5a), 1.36 (3H, s, Me-9), 1.66 (1H, t, J = 6.6 Hz, H-5b), 1.86 (3H, s, Me-10), 2.27 (1H, m, H-4a), 2.90 (1H, m, H-4b), 5.08 (1H, dd, J = 14.0, 10.6 Hz, H-8a), 5.17 (1H, t, J = 14.0, 16.0 Hz, H-8b), 5.93 (1H, dd, J = 10.9, 17.8 Hz, H-7), 6.85 (1H, dt, J = 7.6, 1.5 Hz, H-3); ^{13}C NMR data of the monoterpenoid moiety (CD_3-OD, 150 MHz) δ 12.4 (C-9), 23.4 (C-10), 24.7 (C-4), 41.6 (C-5), 73.8 (C-6), 112.0 (C-8), 128.1 (C-2), 145.2 (C-3), 146.1 (C-7), 169.0 (C-1); ^{1}H NMR data of the cinnamoyl moiety (CD_3OD, 600 MHz) δ 6.78 (1H, d, J = 16.0 Hz, H-2), 6.75 (1H, m, H-3′ and H-5′), 7.45 (1H, br t, J = 8.0 Hz, H-2′ and H-6′), 7.63 (1H, m, H-4′), 7.92 (1H, d, J = 16.0 Hz, H-3); ^{13}C NMR data of the cinnamoyl moiety (CD_3OD, 150 MHz) δ 118.8 (C-2), 129.6 (C-3′, C-5′), 129.6 (C-4′), 131.6 (C-2′, C-6′), 135.7 (C-1′), 148.0 (C-3), 167.5 (C-1); ^{1}H and ^{13}C NMR of the sugar moieties, see Table 1; HR/ESIMS *m/z* 2020.9006 $[M + Na]^+$; MS^n *m/z* 1890 $[M + Na - 130]^+$, 1758 $[M + Na - (130 + 162)]^+$, 1592 $[M + Na - (130 + 162 + 132]^+$, 1124 $[M + Na - (130 + 166 + 600)]^+$, 992 $[M + Na - (130 + 166 + 600 + 132)]^+$, 962 $[M + Na - (130 + 166 + 600 + 162)]^+$, 860 $[M + Na - (130 + 166 + 600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 130 + 166 + 600)]^+$, 520 $[M + Na - (472 + 130 + 166 + 600 + 132)]^+$, 490 $[M + Na - (472 + 130 + 166 + 600 + 162)]^+$, 388 $[M + Na - (472 + 130 + 166 + 600 + 132 + 132)]^+$; *anal*. C 57.64%, H 7.18%, N 0.68%, calcd for $C_{96}H_{143}NO_{43}$, C 57.68%, H 7.21%, N 0.70%.

Compound 2: white solid; $[\alpha]_D^{25}$ −46 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 1; ^{1}H NMR data of the monoterpenoid moiety (CD_3OD, 600 MHz) δ 1.20 (1H, t, J = 6.6 Hz, H-5a), 1.34 (3H, s, Me-9), 1.65 (1H, t, J = 6.6 Hz, H-5b), 1.85 (3H, s, Me-10), 2.28 (1H, m, H-4a), 2.89 (1H, m, H-4b), 5.08 (1H, dd, J = 14.0, 10.0 Hz, H-8a), 5.16 (1H, dd, J = 14.0, 15.8 Hz, H-8b), 5.92 (1H, dd, J = 11.0, 17.8 Hz, H-7), 6.85 (1H, dt, J = 7.6, 1.5 Hz, H-3); ^{13}C NMR data of the monoterpenoid moiety (CD_3OD, 150 MHz) δ 12.2 (C-9), 23.4 (C-10), 25.0 (C-4), 41.5 (C-5), 73.8 (C-6), 111.8 (C-8), 127.8 (C-2), 145.1 (C-3), 146.0 (C-7), 169.1 (C-1); HR/ESIMS *m/z* 1890.8705 $[M + Na]^+$; MS^n *m/z* 1758 $[M + Na - 132]^+$, 1592 $[M + Na - (166 + 132)]^+$, 1124 $[M + Na - (166 + 600)]^+$, 992 $[M + Na - (166 + 600 + 132)]^+$, 962 $[M + Na - (166 + 600 + 162)]^+$, 860 $[M + Na - (166 + 600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 166 + 600)]^+$, 520 $[M + Na - (472 + 166 + 600 + 132)]^+$, 490 $[M + Na - (472 + 166 + 600 + 162)]^+$, 388 $[M + Na - (472 + 166 + 600 + 132 + 132)]^+$; *anal*. C 55.87%, H 7.37%, N 0.76%, calcd for $C_{87}H_{137}NO_{42}$, C 55.91%, H 7.39%, N 0.75%.

Compound 3: white solid; $[\alpha]_D^{25}$ −39 (*c* 0.1, MeOH); ^{1}H NMR data of the aglycon (CD_3OD, 600 MHz) δ 0.78 (3H, s, Me-24), 0.80 (3H, s, Me-26), 0.93 (3H, s, Me-25), 0.95 (3H, s, Me-29), 0.99 (3H, s, Me-23), 1.15 (3H, s, Me-30), 1.35 (3H, s, Me-27), 3.02 (1H, dd, J = 12.8, 4.5 Hz, H-18), 3.16 (1H, dd, J = 11.5, 4.0 Hz, H-3), 4.54 (1H, br m, H-16), 5.08 (1H, dd, J = 11.2, 5.5 Hz, H-21), 5.32 (1H, t, J = 3.5 Hz, H-12); ^{13}C NMR data of the aglycon (CD_3OD, 150 MHz) δ 16.0 (C-25), 17.0 (C-26), 17.8 (C-24), 18.5 (C-6), 19.0 (C-30), 24.0 (C-11), 26.7 (C-2), 27.6 (C-27), 28.0 (C-29), 28.3 (C-23), 34.8 (C-20), 34.2 (C-7), 36.5 (C-22), 36.5 (C-15), 37.8 (C-10), 39.0 (C-4), 39.6 (C-1), 40.6 (C-8), 42.0 (C-14), 41.9 (C-18), 47.7 (C-19), 50.0 (C-17), 48.2 (C-9), 57.2 (C-5), 74.6 (C-16), 77.7 (C-21), 91.0 (C-3), 123.7 (C-12), 144.0 (C-13), 177.8 (C-28); ^{1}H and ^{13}C NMR of the sugar moieties were identical to those of compound **2**; ^{1}H NMR data of the monoterpenoid moiety (CD_3OD, 600 MHz) δ 1.20 (1H, t, J = 6.6 Hz, H-5a), 1.34 (3H, s, Me-9), 1.67 (1H, t, J = 6.6 Hz, H-5b), 1.85 (3H, s, Me-10), 2.27 (1H, m, H-4a), 2.90 (1H, m, H-4b), 5.10 (1H, dd, J = 14.0, 10.5 Hz, H-8a), 5.17 (1H, dd, J = 14.0, 16.0 Hz, H-8b), 5.93 (1H, dd, J = 10.9, 17.8 Hz, H-7), 6.87 (1H, dt, J = 7.6, 1.5 Hz, H-3); ^{13}C NMR data of the monoterpenoid moiety (CD_3OD, 150 MHz) δ 12.6 (C-9), 23.7 (C-10), 24.7 (C-4), 41.9 (C-5), 73.8 (C-6), 112.1 (C-8), 128.4 (C-2), 145.3 (C-3), 146.3 (C-7), 169.3 (C-1); HR/ESIMS *m/z* 1906.8583 $[M + Na]^+$, MS^n *m/z* 1774 $[M + Na - 132]^+$, 1722 $[M + Na - 184]^+$, 1140 $[M + Na - (166 + 600)]^+$, 1008 $[M + Na - (166 + 600 + 132)]^+$, 876 $[M + Na - (166 + 600 + 132 + 132)]^+$, 652 $[M + Na - (488 + 166 + 600)]^+$, 520 $[M + Na - (488 + 166 + 600 + 132)]^+$, 490 $[M + Na - (488 + 166 + 600 + 162)]^+$, 388 $[M + Na - (488 + 166 + 600 + 132 + 132)]^+$; *anal*. C 57.38%, H 7.29%, N 0.71%, calcd for $C_{87}H_{137}NO_{43}$, C 55.43%, H 7.33%, N 0.74%.

Compound 4: white solid; $[\alpha]_D^{25}$ −29 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H NMR data of the cinnamoyl moiety (CD_3OD, 600 MHz) δ 6.78 (1H, d, J = 16.0 Hz, H-2), 7.45 (1H, br t, J = 8.0 Hz, H-2′ and H-6′), 7.63 (1H, m, H-4′), 6.68 (1H, m, H-3′ and H-5′), 7.92 (1H, d, J = 16.0 Hz, H-3); ^{13}C NMR data of the cinnamoyl moiety (CD_3OD, 150 MHz) δ 118.8 (C-2), 129.8 (C-3′, C-5′), 129.8 (C-4′), 131.6 (C-2′, C-6′), 135.9 (C-1′), 148.1 (C-3), 167.8 (C-1); ^{1}H and ^{13}C NMR of the sugar moieties, see Table 1; ESIMS and MS^n *m/z* 1854 $[M + Na]^+$, 1812 $[M + Na - 42]^+$, 1724 $[M + Na - 130]^+$, 1124 $[M + Na - (130 + 600)]^+$, 992 $[M + Na - (130 + 600 + 132)]^+$, 962 $[M + Na - (130 + 600 + 162)]^+$, 860 $[M + Na - (130 + 600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 130 + 600)]^+$, 520 $[M + Na - (472 + 130 + 600 + 132)]^+$, 490 $[M + Na - (472 + 130 + 600 + 162)]^+$, 388 $[M + Na - (472 + 130 + 600 + 132 + 132)]^+$; *anal*. C 56.32%, H 7.05%, N 0.74%, calcd for $C_{86}H_{129}NO_{41}$, C 56.35%, H 7.09%, N 0.76%.

Compound 5: white solid; $[\alpha]_D^{25}$ −28 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; ESIMS and MS^n *m/z* 1724 $[M + Na]^+$, 1124 $[M + Na - 600]^+$, 992 $[M + Na - (600 + 132)]^+$, 962 $[M + Na - (600 + 162)]^+$, 860 $[M + Na - (600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 600)]^+$, 520 $[M + Na - (472 + 600 + 132)]^+$, 490 $[M + Na - (472 + 600 + 162)]^+$, 388 $[M + Na - (472 + 600 + 132 + 132)]^+$; *anal*. C 54.26%, H 7.27%, N 0.79%, calcd for $C_{77}H_{123}NO_{40}$, C 54.31%, H 7.28%, N 0.82%.

Compound 6: white solid; $[\alpha]_D^{25}$ −25 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; ESIMS and MS^n *m/z* 1682 $[M + Na]^+$, 1124 $[M + Na - 558]^+$, 992 $[M + Na - (558 + 132)]^+$, 962 $[M + Na - (558 + 162)]^+$, 860 $[M + Na - (558 + 132 + 132)]^+$, 652 $[M + Na - (472 + 558)]^+$, 520 $[M + Na -$

$(472 + 558 + 132)]^+$, 490 $[M + Na - (472 + 558 + 162)]^+$, 388 $[M + Na - (472 + 558 + 132 + 132)]^+$; *anal*. C 54.20%, H 7.29%, N 0.81%, calcd for $C_{75}H_{121}NO_{39}$, C 54.24%, H 7.34%, N 0.84%.

Compound 7: white solid; $[\alpha]_D^{25}$ −23 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **3**; ^{1}H and ^{13}C NMR of the sugar moieties were identical to those of compound **5**; ESIMS and MS^n *m/z* 1740 $[M + Na]^+$, 1140 $[M + Na - 600]^+$, 1008 $[M + Na - (600 + 132)]^+$, 978 $[M + Na - (600 + 162)]^+$, 876 $[M + Na - (600 + 132 + 132)]^+$, 652 $[M + Na - (488 + 600)]^+$, 520 $[M + Na - (488 + 600 + 132)]^+$, 490 $[M + Na - (488 + 600 + 162)]^+$, 388 $[M + Na - (488 + 600 + 132 + 132)]^+$; *anal*. C 53.77%, H 7.19%, N 0.77%, calcd for $C_{77}H_{123}NO_{41}$, C 53.81%, H 7.21%, N 0.81%.

Compound 8: white solid; $[\alpha]_D^{25}$ −11 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H NMR data of the cinnamoyl moiety (CD_3OD, 600 MHz) δ 6.78 (1H, d, $J = 16.0$ Hz, H-2), 7.45 (1H, br t, $J = 8.0$ Hz, H-2′ and H-6′), 7.63 (1H, m, H-4′), 6.67 (1H, m, H-3′ and H-5′), 7.92 (1H, d, $J = 16.0$ Hz, H-3); ^{13}C NMR data of the cinnamoyl moiety (CD_3OD, 150 MHz) δ 119.0 (C-2), 129.6 (C-3′, C-5′), 129.8 (C-4′), 131.9 (C-2′, C-6′), 136.2 (C-1′), 148.2 (C-3), 168.0 (C-1); ^{1}H and ^{13}C NMR of the sugar moieties, see Table 3; ESIMS and MS^n *m/z* 1854 $[M + Na]^+$, 1724 $[M + Na - 130]^+$, 1124 $[M + Na - (130 + 600)]^+$, 992 $[M + Na - (130 + 600 + 132)]^+$, 962 $[M + Na - (130 + 600 + 162)]^+$, 860 $[M + Na - (130 + 600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 130 + 600)]^+$, 520 $[M + Na - (472 + 130 + 600 + 132)]^+$, 490 $[M + Na - (472 + 130 + 600 + 162)]^+$, 388 $[M + Na - (472 + 130 + 600 + 132 + 132)]^+$; *anal*. C 56.30%, H 7.06%, N 0.73%, calcd for $C_{86}H_{129}NO_{41}$, C 56.35%, H 7.09%, N 0.76%.

Compound 9: white solid; $[\alpha]_D^{25}$ −9 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data of the aglycon were superimposable on those reported for **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 3; ESIMS and MS^n *m/z* 1724 $[M + Na]^+$, 1124 $[M + Na - 600]^+$, 992 $[M + Na - (600 + 132)]^+$, 962 $[M + Na - (600 + 162)]^+$, 860 $[M + Na - (600 + 132 + 132)]^+$, 652 $[M + Na - (472 + 600)]^+$, 520 $[M + Na - (472 + 600 + 132)]^+$, 490 $[M + Na - (472 + 600 + 162)]^+$, 388 $[M + Na - (472 + 600 + 132 + 132)]^+$; *anal*. C 54.18%, H 7.26%, N 0.78%, calcd for $C_{77}H_{123}NO_{40}$, C 54.31%, H 7.28%, N 0.82%.

Acid Hydrolysis of Compounds 1−9. A solution of each compound **1**−**9** (2.0 mg) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. On cooling, the solution was evaporated under a stream of N_2. Each 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 l-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 deg/min. Peaks of the hydrolyzate were detected by comparison with retention times of authentic samples of D-glucose, D-xylose, L-arabinose, and d-apiose (Sigma-Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

Basic Hydrolysis of Compound 1. Compound **1** (0.5 mg) in 0.5 M KOH (0.5 mL) was heated at 110 °C in a reaction vial for 2 h. The reaction mixture was cooled, adjusted to pH 7 with 2 M HCl, and injected in the LC-MS system. Reaction products were identified on the basis of their molecular weight, fragmentation pattern, and retention time.

Hydrolysis of Compounds 1−3. A solution of **1** (5 mg), **2** (10 mg), and **3** (8 mg) in 1% di-$NAHCO_3$−H_2O was refluxed for 1 h, respectively. The reaction mixture was cooled, adjusted to pH 7 with 1 M HCl, and subjected to chromatography on a silica gel column eluted with $CHCl_3$−MeOH (100:0 to 1:1) to afford the MT moiety.[16]

Antiproliferative Activity Assay. J774.A1 (murine monocyte/macrophage), WEHI-164 (murine fibrosarcoma), and HEK-293 (human epithelial kidney) cells were grown as reported previously.[25] 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 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.[26,27] 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 % dead cells = 100 − (OD treated/OD control) × 100.

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