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Modified Jatrophane Diterpenes as Modulators of Multidrug Resistance from *Euphorbia Dendroides* L.

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Abstract—The new diterpenoids terracinolides J-L (**1**–**3**), 13α-OH terracinolide F (**8**), abeodendroidin F (**11**) and epiabeodendroidin F (**12**) have been identified from *Euphorbia dendroides* L. The new compounds and six co-occurring known terracinolides were tested as inhibitors of the drug-efflux activity of P-glycoprotein from cancer cells. The results were used to extend the structure–activity relationships established for this class of compounds highlighting the relevance of substitution at positions 2, 3, 6, and 15 and disclosing a remarkable tolerance toward connectivity changes in the terpenoid core.

Introduction

Euphorbia dendroides L. is a tree-like semi-succulent spurge growing on rocky coastal places in the Western Mediterranean area and especially in Sardinia.[1] This attractive plant can reach 2 m in height and is an essential element of the Mediterranean landscape, for which it has gained a sort of icon state, not unlike Edelweiss for the Alps.[1] *E. dendroides* has a rich ethnopharmacology, already documented in the Greek and Roman medical literature. Thus, like other spurges, the plant was used to remove warts, as a fish poison, and as a cathartic, its properties being substantially similar to those of *E. characias* L., one of the most offensive spurges according to the ancients.[2] Despite its popularity and abundance, *E. dendroides* has been surprisingly overlooked from a phytochemical standpoint, and the major focus of scientific investigation on this species has long been limited to its use as a plentiful source of biomass for the production of fuel.[3] On the other hand, spurges are prolific producers of unique diterpenoids[4] of great biomedical relevance.[5] This has spurred our interest in *E. dendroides*, from which we have recently reported the isolation of nine new jatrophane diterpenoids of relevance as revertant agents due to their powerful inhibitory activity on P-glycoprotein (Pgp)-mediated transport.[6] In particular, one of the isolated metabolites, euphodendroidin D, showed highly potent activity outperforming cyclosporin A by a factor of 2 in the inhibition of Pgp-mediated daunomycin transport. We now complete the characterization of the diterpenoid fraction of *E. dendroides* by describing the structural elucidation and biological evaluation of 12 further constituents of the modified jatrophane type. In particular, latex of *E. dendroides* yielded 10 terracinolides, four of which, terracinolides J, K, L (**1**–**3**) and 13α-OH terracinolide F (**8**), are novel compounds and two *abeo*-jatrophanes, named abeodendroidin F (**11**) and epiabeodendroidin F (**12**), both of them new molecules (Chart 1).

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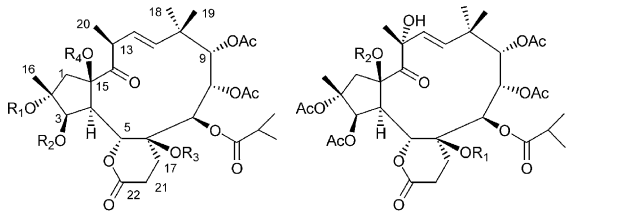
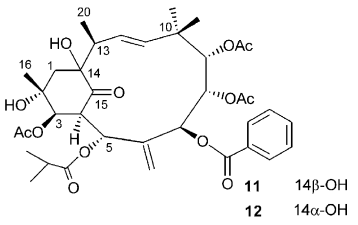


	R_1	R_2	R_3	R_4
1	Ac	Ac	iBu	H
2	H	Ac	Ac	Ac
3	H	Ac	iBu	Ac
4	Ac	Ac	Ac	Ac
5	Ac	H	Ac	Ac
6	Ac	Ac	iBu	Ac
7	Ac	H	iBu	Ac

	R_1	R_2
8	iBu	Ac
9	Ac	Ac
10	Ac	H



11 14β-OH
12 14α-OH

Chart 1. Chemical structures of **1**–**12**.

Results and Discussion

Latexes obtained from three different collections of *E. dendroides* L. from the island of Sardinia (Italy) were diluted with EtOAc and filtered on silica gel in order to eliminate gummy polar compounds.

The soluble material was purified by repeated silica gel chromatographies to give compounds **1**–**12** (Chart 1).

Compounds **4**–**7** and **9**–**10** were identified as terracinolides B, C, F, H, and 13α-OH terracinolide B and G, respectively, by comparison of their MS and NMR data with those reported for the original compounds isolated from *E. terracina* and *E. segetalis*.[7–10] The remaining compounds are instead new.

Terracinolide J (**1**) gave a pseudomolecular ion peak at *m/z* 767.3488 ($[M+H]^+$) in the positive ions HRFABMS, corresponding to the molecular formula $C_{38}H_{54}O_{16}$. Both the above mass data and 1D (^1H and ^{13}C) NMR spectra of **1** (Tables 1 and 2) suggested that this compound was a diterpenoid polyester. In particular, the presence of three methyl signals (δ 0.93, 1.20, 1.58, singlets), an isolated methylene group [δ_H 2.80 (d, 17 Hz) and 2.11 (d, 17 Hz)], one ketone carbonyl (δ_C 213.2), one *trans*-disubstituted double bond [δ_C 129.5 and 136.0; δ_H 5.40 (dd, 16, 10 Hz) and 6.07 (d, 16 Hz)], and seven ester carbonyls [δ_C 170.1, 170.2 (×3), 172.3, 174.7, 176.7] was disclosed. Six of these acyl groups were easily identified as four acetates and two isobutyrate groups by routine inspection of NMR data. The combined analysis of the 2D COSY and HSQC spectra of **1** sorted the protons of the diterpene core structure into four spin systems (C-3–C-5, C-7–C-9, C-11–C-13/C-20, and C-17–C-21), next connected by the analysis of the $^{2,3}J_{C-H}$ correlations in the 2D HMBC spectrum, as summarized in Figure 1. In this way, a 17-ethyl bis-homojatrophane framework was assigned to **1**, a skeleton first found in *E. terracina*,[7] which according to Marco et al. could arise through opening of a 5,17-epoxide by nucleophilic attack of a C_2 unit (acetate or malonate) followed by lactone ring closure.[8] The next step of structural determination was the location of the functional groups on the oxygenated carbons 2, 3, 5, 6, 7, 8, 9, and 15. This last carbon was bound to a free hydroxyl as clear by the detection of a HMBC cross-peak of the exchangeable proton at δ 4.11 with C-15. 2D HMBC measurements were instrumental to locate four of the six acyl groups, establishing the linkage of three acetate groups at C-3, C-8, and C-9, respectively, and of the isobutyrate group at C-7 (see Fig. 1). On the other hand, since the two remaining ester groups (one acetate and one isobutyrate) are linked to unprotonated carbons (C-2 and C-6), $^{2,3}J_{C-H}$ correlations could not be used to locate them. To solve this issue, analysis of spatial proximities inferred from a ROESY experiment (Fig. 2) was used.

Indeed, the unassigned methyl acetate singlet showed ROESY cross-peak with CH_3-16, strongly pointing for its location at C-2 and thus placing the isobutyrate group at C-6. The relative configuration of terracinolide J (**1**) was investigated by measurement of scalar and dipolar couplings (Fig. 2) as well as by comparison with literature data.[7–9] Thus, the OH-15 signal showed a dipolar correlation with H-5 and with the isobutyrate methine at C-6, while the acetate at C-2 was correlated to H-4 and H-3. These data secured the relative configuration of the five-membered ring and pointed to a *trans*-oriented A/B ring junction. Furthermore, as already reported in the literature,[11] the very small values of the coupling constants H-7/H-8 and H-8/H-9 are consistent with a *cis* orientation for H-8 and H-9 and a *trans* orientation for H-7 and H-9. The ROESY cross peaks of H-4 with H-7 and H-13 and of H_3-19 with H-7 completed the relative geometry of the twelve-membered ring of terracinolide J (**1**). The relative configuration of **1** has been found thus identical to that of all known terracinolides, and also the same absolute configuration has been assumed.

Terracinolide K (**2**), $C_{36}H_{50}O_{16}$ by HR-FABMS, is closely related to terracinolide J, the only difference being the replacement of an isobutyrate by an acetate. Therefore, the structural elucidation of terracinolide K (**2**), based on inspection of 2D NMR spectra (COSY, HSQC, and HMBC), was guided by comparison with parallel spectral data of terracinolide J (**1**). Long-range correlations in the HMBC spectrum established the linkage of three acetates at C-3, C-8 and C-9, respectively, and of the isobutyrate at C-7. Consequently, two of the three remaining oxygenated carbons (the unprotonated C-2, C-6, and C-15) must link acetate groups. Since the free hydroxyl group was situated at C-2 on the basis of the HMBC cross-peak of OH-2 (δ 2.18) with C-2, the two acetoxy groups were located at C-6 and C-15, respectively. Thus, terracinolide K (**2**), is the first terracinolide bearing a free hydroxyl at C-2 isolated to date. Stereochemical details of compound **2** were investigated through ROESY measurement (correlations H-3/H-7, H-3/OH-2, H-3/H-1α, H-1α/H-4, H-4/H-13, H-8/H-9, H-8/isobutyrate protons, and H-5/15-OAc), in accordance with the same relative configuration above

Table 1. ^{1}H NMR data (500 MHz) of new terracinolides (**1**–**3**, and **8**) and abeojatrophanes (**11**–**12**) in $CDCl_3$

Pos.		**1**	**2**	**3**	**8**	**11**	**12**
		δ, mult., *J* in Hz	δ, mult., *J* in Hz	δ, mult., *J* in Hz	δ, mult., *J* in Hz	δ, mult., *J* in Hz	δ, mult., *J* in Hz
1α		2.80, d, 17	2.74, d, 17	2.73, d, 17	4.04, d, 18	2.23, d, 17	2.48, d, 17
1β		2.11, d, 17	2.52, d, 17	2.48, d, 17	2.75, d, 18	1.58, d, 17	1.71, d, 17
3		5.79, d, 3.5	5.21, d, 3.5	5.20, d, 3.5	5.49, d, 3	5.51, d, 2	5.26, d, 2
4		3.78, dd, 9, 3.5	4.05, dd, 9, 3.5	4.04, dd, 9, 3.5	3.85, dd, 8.5, 3	4.22, dd, 12, 2	4.80, dd, 12, 2
5		5.48, d, 9	5.57, d, 9	5.69, d, 9	5.63, d, 8.5	5.12, d, 12	5.55, d, 12
7		6.18, s	6.13, s	6.15, s	5.89, s	6.32, s	6.33, s
8		5.52, s	5.65, s	5.62, s	5.55, s	5.21, s	5.20, s
9		4.87, s	4.87, s	4.88, s	4.90, s	4.93, s	4.90, s
11		6.07, d, 16	5.95, d, 16	5.95, d, 16	6.08, d, 17	6.13, d, 16	6.09, d, 16
12		5.40, dd, 16, 10	5.42, dd, 16, 10	5.42, dd, 16, 10	5.88, d, 17	5.30, dd, 16, 10	5.24[a]
13		4.00, dq, 10, 6.5	4.42, dq, 10, 6.5	4.40, dq, 10, 6.5		3.06, dq, 10, 7	3.70, dq, 10, 7
16		1.58, s	1.32, s	1.32, s	1.50, s	1.15, s	1.20, s
17α		2.41[a]	2.45[a]	2.42[a]	2.58[a]	5.73, s	5.71, s
17β		1.66[a]	1.87[a]	1.74[a]	1.70, m	5.42, s	5.40, s
18		0.93, s	0.92, s	0.92, s	0.98, s	0.79, s	0.80, s
19		1.20, s	1.32, s	1.25, s	1.29, s	1.14, s	1.10, s
20		1.32, d, 6.5	1.27, d, 6.5	1.25[a]	1.56, s	0.95, d, 7	1.11, d, 7
21α		2.42[a]	2.44[a]	2.43[a]	2.46, m		
21β		3.30, dt, 15, 6.5	3.29, dt, 15, 6.5	3.29, dt, 15, 6.5	3.31, m		
2-OH			2.18, s	2.28, s		2.07, s	2.45, s
13-OH					3.46, s		
14-OH						3.81, s	3.92, s
15-OH		4.11, s					
Ac		2.00, s	1.98, s	1.98, s	2.00, s	1.43, s	1.40, s
		2.01, s	2.04, s	2.03, s	2.03, s	1.96, s	1.97, s
		2.02, s	2.04, s	2.03, s	2.06, s	2.16, s	2.12, s
		2.29, s	2.10, s	2.07, s	2.06, s		
			2.15, s		2.21, s		
IBu	2	2.58, hept, 7	2.58, hept, 7	2.58, hept, 7	2.62, hept, 7	1.81, hept, 7	1.78, hept, 7
	3	1.25, d, 7	1.25, d, 7	1.26, d, 7	1.24, d, 7	0.61, d, 7	0.78, d, 7
	4	1.19, d, 7	1.19, d, 7	1.20, d, 7	1.21, d, 7	0.82, d, 7	0.58, d, 7
IBu	2′	2.35, hept, 7		2.51, hept, 7	2.58, hept, 7		
	3′	1.25, d, 7		1.25, d, 7	1.24, d, 7		
	4′	1.25, d, 7		1.20, d, 7	1.20, d, 7		
Bz							
	AA′					8.06, d, 7.5	8.05, d, 7.5
	BB′					7.44, t, 7.5	7.40, t, 7.5
	C					7.57, d, 7.5	7.52, d, 7.5

[a]Overlapped with other signals.

established for terracinolide J (**1**). Finally, the coupling constant H-11/H-12 ($J = 16$ Hz) indicated the *E* geometry of the endocyclic double bond.

Terracinolide L (**3**) had the same molecular formula of terracinolide J (**1**) (HR-FABMS). Both ^{13}C and ^{1}H NMR spectra of **3** (Tables 1 and 2) appeared practically superimposable to parallel spectra of terracinolide K (**2**), differing only for the lack of C/H resonances attributable to an acetate group, replaced by signals of an additional isobutyrate residue. The carbon and proton chemical shifts of **3** were assigned by COSY, HSQC and HMBC measurements, that indicated the linkage of acetoxyls at C-3, C-8, and C-9, an isobutyrate at C-7, and a free hydroxyl at C-2. Consequently, only one acetate and one isobutyrate remained to be located at the unprotonated oxygenated carbons C-6 and C-15. ROESY experiment was instrumental to solve this problem; indeed, cross-peak of H_3-16 with the unassigned methyl acetate singlet strongly suggested that this group was located at C-15 and, consequently, the isobutyrate group at C-6. Finally, ROESY experiment secured that **3** has the same relative configuration of all known terracinolides.

The ^{1}H and ^{13}C NMR spectra of **8** (Tables 1 and 2), $C_{40}H_{56}O_{18}$ by HR-FABMS, analyzed through inspection of 2D NMR experiments (COSY, HSQC, and HMBC), showed that this compound was a terracinolide linking five acetate and two isobutyrate ester groups, located by 2D NMR experiments as detailed before. The NMR spectra of **8** were very similar to those of terracinolide F (**6**),[8] also isolated from the diterpenoid fraction of *E. dendroides*, with differences rationalisable by the presence of an additional hydroxyl at C-13 in **8**. Thus, the signal of H-13 was missing in the ^{1}H NMR spectra of **8**, where the CH_3-20 resonated as a singlet (δ 1.56) and H-12 as a doublet (δ 5.88). Taken together, these observations suggest that **8** is 13-OH terracinolide F. The relative configuration of the chiral centers of **8** has been determined through ROESY and coupling constant analysis and revealed to be identical to that of all the other terracinolides. In particular, ROESY cross-peak H-4/OH-13 indicated the α orientation of the OH group at C-13.

Table 2. ^{13}C NMR data (125 MHz) of new terracinolides (**1**–**3**, **8**) and abeojatrophanes (**11**–**12**) in $CDCl_3$

Pos.		1	2	3	8	11	12
1		52.31 t	50.28 t	52.53 t	45.51 t	41.50 t	46.22 t
2		87.43 s	90.59 s	90.76 s	86.30 s	72.20 s	71.18 s
3		79.14 d	82.04 d	81.33 d	81.05 d	75.01 d	78.35 d
4		44.65 d	45.40 d	44.39 d	46.79 d	50.01 d	45.58 d
5		72.27 d	71.66 d	71.86 d	72.23 d	72.10 d	72.24 d
6		79.91 s	79.89 s	78.20 s	80.81 s	140.15 s	139.95 s
7		66.56 d	66.15 d	66.73 d	66.63 d	65.51 d	65.34 d
8		67.24 d	66.48 d	67.33 d	67.55 d	71.25 d	71.34 d
9		81.33 d	82.04 d	81.12 d	81.22 d	81.70 d	81.02 d
10		39.94 s	42.24 s	39.51 s	39.66 s	40.11 s	39.51 s
11		136.00 d	134.56 d	134.77 d	131.01 d	135.79 d	135.34 d
12		129.50 d	131.31 d	131.94 d	133.43 d	128.80 d	128.99 d
13		43.62 d	41.19 d	41.71 d	82.05 d	43.00 d	43.16 d
14		213.19 s	204.87 s	205.19 s	204.51 s	78.22 s	79.60 s
15		84.48 s	78.38 s	78.41 s	92.22 s	211.11 s	211.13 s
16		18.32 q	22.64 q	24.30 q	19.36 q	15.99 q	15.47 q
17		25.12 t	25.28 t	26.24 t	26.19 t	125.25 t	125.20 t
18		26.22 q	25.95 q	27.03 q	26.03 q	27.55 q	22.62 q
19		23.60 q	22.57 q	22.40 q	22.47 q	23.36 q	27.34 q
20		21.50 q	20.46 q	20.50 q	29.55 q	17.65 q	27.00 q
21		28.83 t	28.22 t	29.30 t	28.71 t		
22		172.31 s	172.29 s	171.29 s	172. 02 s		
Ac	1	170.10 s	169.85 s	169.95 s	170.40 s	170.99 s	170.43 s
		170.22 s	169.67 s	169.94 s	169.83 s	170.40 s	170.19 s
		170.22 s	169.36 s	169.37 s	169.82 s	170.25 s	169.54 s
		170.22 s	169.37 s	169.02 s	168.85 s		
			169.02 s		168.84 s		
	2	21.51 q	22.46 q	22.04 q	22.41 q	20.98 q	20.65 q
		20.92 q	22.00 q	21.94 q	22.33 q	20.72 q	20.12 q
		20.87 q	21.83 q	21.95 q	21.39 q	21.55 q	21.08 q
		20.79 q	21.84 q	21.88 q	21.02 q		
			20.84 q		20.55 q		
iBu	1	174.66 s	174.72 s	176.70 s	175.55 s	176.00 s	175.49 s
		176.65 s		175.47 s	176.30 s		
	2	35.13 d	35.05 d	34.99 d	34.40 d	34.21 d	33.64 d
		34.69 d		34.23 d	35.01 d		
	3	19.88 q	19.11 q	19.60 q	19.11 q	18.15 q	17.89 q
		19.25 q		18.51 q	18.96 q		
	4	18.83 q	16.91 q	17.81 q	18.82 q	18.99 q	18.21 q
		18.05 q	17.82 q	18.50 q			
Bz						166.01 s	165.12 s
						131.10 s	130.40 s
						130.00 d	129.71 d
						129.11 d	128.54 d
						133.99 d	133.36 d

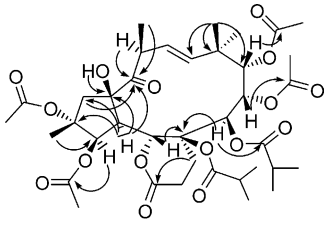


Figure 1. Selected HMBC correlations exhibited by terracinolide J (**1**).

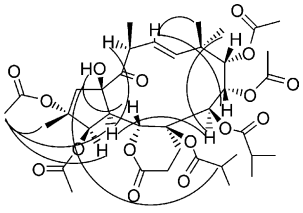


Figure 2. Selected ROESY correlations exhibited by terracinolide J (**1**).

Two polyesterified rearranged jatrophanes (**11** and **12**) were also isolated, and their structures elucidated on the basis of NMR spectral analysis and comparison with jatrophane NMR data. HR-FAB measurements revealed that compound **11** possesses the molecular formula $C_{37}H_{48}O_{13}$. The same molecular formula has been observed for euphodendroidin F (**13**, Fig. 3), a jatrophane compound that we isolated from the same source.[6] Comparison of ^1H and ^{13}C NMR data (Tables 1 and 2) of **11** with those of **13** revealed the same type, number, and location of the ester residues (three acetates, one isobutyrate, one benzoate) and all other functional groups (one ketone, two hydroxyl groups, one *trans*-disubstituted double bond, one *exo*-cyclic double bond). On the other hand, HMBC correlations showed marked differences in the carbons involved and around the ring junction. Thus, the cross peaks H-1/C-13, H-3/C-15, and H-20/C-14 suggested that compound **11** could derive from **13** through the α-ketol type rearrangement already described for a similar compound.[12] Although this kind of rearrangement could potentially occur in all the 14-keto-15-hydroxy jatrophanes, to our knowledge, compound **11**, that we have named abeodendroidin F, represents only the second *abeo*-jatrophane described in the literature.

The relative configuration of the macrocyclic ring of **11** was secured by 2D ROESY cross-peaks as detailed previously for the new terracinolides, and was identical to that of euphodendroidin F (**13**). On the other hand, the cross peaks of H-4 with H-13, H-3 and OH-2 and of CH_3-16 with $OCOC\underline{H}_3$-3 dictate the relative configuration of ring A as reported in the figure, thus completing structural characterization of abeodendroidin F (**11**).

HR-FABMS of compound **12** indicated the same molecular formula of abeodendroidin F (**11**) and, analogously, analysis of ^1H and ^{13}C NMR spectra of **12** (Tables 1 and 2), accomplished through the use of 2D NMR experiments, indicated the same gross structure of compound **11**. The only way for explaining these results is that the two molecules differ for the stereochemistry of one (or more) chiral carbon. Actually, the proton resonance of H-4 is considerably different in the two compounds (δ 4.22 in **11** and δ 4.80 in **12**) and this downfield shift is suggestive of a *cis*-orientation of H-4 with both OH-2 and OH-14 in **12**. Accordingly, while the ROESY spectrum of **12** suggested the standard configuration around the macrocyclic ring, it showed correlations of H-4 with both OH-14 and OH-2 and of $OCOC\underline{H}_3$-3 with CH_3-16, thus confirming the assignment of **12** as the C-14 epimer of abeodendroidin F (**11**).

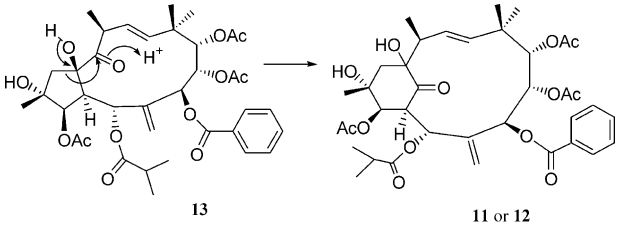


Figure 3. Proposed rearrangement of euphodendroidin F (**13**) to yield compounds **11** and **12**.

Compounds **11** and **12** are the result of the two alternative courses for the α-ketol rearrangement of euphodendroidin F (**13**) (Fig. 3); therefore, we became interested in mimicking this reaction under laboratory conditions. To this aim, the stability of **13** was investigated under a variety of acidic and basic conditions which could promote the reaction. In accordance with a previous finding on a related ketolic jatrophane,[12] **13** was stable under acidic conditions, while treatment with non-nucleophilic bases (NaH, DBU, KHMDS) gave complex mixtures. On the other hand, while optimizing the TLC conditions for the separation of compounds **11**–**13**, we serendipitously observed that **13** was selectively converted to **12** during elution on neutral alumina. While column chromatography on neutral alumina of **13** resulted in a rather poor conversion **12** (<10%) due to irreversible adsorption, the reaction could be carried out in preparative scale by overnight stirring a solution of **13** in EtOAc with a 10-fold w/w excess of neutral alumina. After gravity column chromatography on silica gel, the rearranged compound **12** was obtained in ca. 60% yield and uncontaminated (^{1}H NMR analysis) by its isomer **11**. Since neutral alumina had not been employed at any stage in the isolation of **12** from *E. dendroides*, this compound should be considered as a 'bona fide' natural product. The diastereoselectivity of the ketol rearrangement is puzzling, and worth a systematic investigation that, owing to the paucity of compound **13** could not be carried out.

The efficiency of terracinolides and abeojatrophanes to inhibit P-glycoprotein-mediated daunomycin efflux was monitored by intracellular accumulation of the drug. Terracinolide H (**7**) was found to be a highly potent inhibitor, even more efficient (138±27%) than cyclosporin A (Cs A), a conventional modulator known as the most active compound and taken here as the reference (Fig. 4). Structure–activity relationships among the eleven

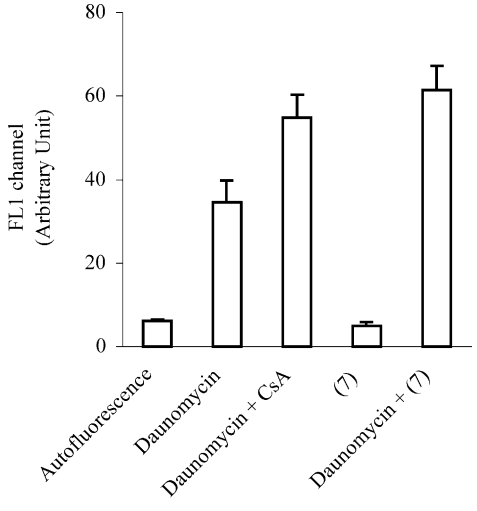


Figure 4. Accumulation of daunomycin in P-glycoprotein-overexpressing human K562/R7 leukemic cells, as monitored by flow cytometry. The cells were exposed to the drug for 1 h in the absence or presence of either cyclosporin A (Cs A) or terracinolide H (**7**), and quickly washed in ice-cold PBS; intracellular drug concentrations were compared by the shift in fluorescence (FL channel). The results represent the mean±SD of at least three independent experiments.

Table 3. Inhibition by terracinolides (**1**–**10**) and abeojatrophanes (**11** and **12**), relatively to cyclosporine A (CsA), of cellular P-glycoprotein-mediated daunomycin efflux monitored by intracellular drug accumulation[a]

Compd	Subst. at C-2	Subst. at C-3	Subst. at C-6	Subst. at C-13	Subst. at C-15 (C-14 for **11** and **12**)	Daunomycin-efflux inhibition compared to CsA (%)
1	OAc	OAc	OiBu	H	OH	101±19
2	OH	OAc	OAc	H	OAc	58±6
3	OH	OAc	OiBu	H	OAc	0
4	OAc	OAc	OAc	H	OAc	22±8
5	OAc	OH	OAc	H	OAc	52±11
6	OAc	OAc	OiBu	H	OAc	0
7	OAc	OH	OiBu	H	OAc	138±27
8	OAc	OAc	OiBu	OH	OAc	Not assayed
9	OAc	OAc	OAc	OH	OAc	27±13
10	OAc	OAc	OAc	OH	OH	102±12
11	OH	OAc	—	H	β-OH	36±6
12	OH	OAc	—	H	α-OH	53±11

[a]The increase in daunomycin accumulation produced by each compound was determined under the same conditions as in Figure 4. The effect produced by CsA was taken as 100% (±15%).

studied compounds showed the effects of substitutions at positions 3, 6 and 15 (Table 3). Firstly, the hydroxyl group at position 3 appeared of major importance since its substitution by an acetyl in (**6**) completely abolished the inhibitory effect. A less efficient effect was produced when the hydroxyl group was positioned at position 15 as in (**1**) (101±19% inhibition). On the other hand, an hydroxyl group at position 2 dramatically decrease the activity (**3**) (no inhibition). In addition, presence of an hydroxyl group at position 13 had no effect when comparing either (**9**) to (**4**) (27±13% vs 22±8%), or (**10**) to (**1**) (102±12% vs 101±19%). Substitution at position 6 gave variable effects depending on the position of the free hydroxyl group on ring A: isobutyl in (**7**) was better than acetyl in (**5**) with hydroxyl at position 3 (138±27% vs 52±11%), whereas acetyl in (**2**) was better than isobutyl (**3**) with hydroxyl at position 2 (58±6% vs no inhibition). Finally, in the abeojatrophane series, when a hydroxyl group was already present at position 2, the addition of a second one at position 14 was better under the α in (**12**) than the β (**11**) configuration (53±11% inhibition vs 36±6%).

Conclusion

The biological evaluation of the modified jatrophanes **1**–**12** confirms the relevance of the substitution at C-3 evidenced by a previous investigation on jatrophanes.[6] The new main information is that the revertant activity of terracinolides and abeojatrophanes is strongly affected by the presence of a free hydroxyl group, with the following ranking of position: 3>15>13>2. In addition, substitution at position 6 affect the inhibitory ability in a way that dramatically depends on the location of the free hydroxyl group. Taken together, these observations suggest that jatrophanes and modified jatrophanes share a common gross pharmacophore, which is dramatically affected by changes of the oxygenation pattern, but surprisingly tolerant in terms of

modifications of connectivity. Owing to the bulk availability of *E. dendroides* and the relatively easy isolation of the major constituents of its diterpenoid fraction, this plant qualifies as an interesting source of bioactive chemotypes for detailed structure–activity studies on an emerging new class of powerful P-glycoprotein inhibitors.

Experimental

General experimental procedures

FABMS (recorded in a glycerol matrix) were measured on a Prospec Fisons mass spectrometer. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10-cm microcell. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX-500 spectrometer. Chemical shifts were referred to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). ^{1}H connectivities were determined by using COSY experiments; One-bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HSQC pulse sequence Two and three bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HMBC experiments, optimized for $^{2\text{-}3}J_{CH}$ of 8 Hz. MPLC was performed on a Büchi 861 apparatus using silica gel (230–400 mesh) columns. HPLC in isocratic mode was performed on a Varian apparatus equipped with an RI-3 refractive index detector using LUNA (Phenomenex) SI60 and RP18 columns.

Plant material

The latexes of three different samples of *E. dendroides* were collected in the surroundings of Arzana (Nuoro, Italy) in March 1999, February 2000 and March 2000. The plant material was identified by MB and a voucher specimen has been deposited at the Dipartimento di Scienze Botaniche, Cagliari (Italy).

Extraction and isolation

Each sample of latex (ca. 10 mL each) was diluted with EtOAc (ca. 5 mL/mL of latex) and then filtered on TLC-grade silica gel. After washing with brine, the organic phase was evaporated to yield a gummy material (collection March 1999: 3 g; February 2000: 2.1 g; March 2000: 0.9 g). The organic extract of the first collection was chromatographed by MPLC on silica gel column (230–400 mesh) using a gradient system from hexane to EtOAc. A fraction (497.9 mg) eluted in hexane/EtOAc 1:1 contained diterpenoids of the jatrophane family, and was first separated by MPLC (from hexane/EtOAc 7:3 to hexane/EtOAc 2:8), affording two jatrophane subfractions: A (264.5 mg) eluted in hexane/EtOAc 1:1 and B (157.7) eluted in hexane/EtOAc 4:6. Fraction A was purified by HPLC (hexane/EtOAc 65:35) to give terracinolide F (**6**, 30 mg), terracinolide B (**4**, 20 mg), terracinolide L (**3**, 1,5 mg), epiabeodendroidin F (**12**, 50 mg) and abeodendroidin F (**11**, 35 mg). Fraction B was purified by HPLC (hexane/EtOAc 6:4) to give terracinolide H (**7**, 2 mg) and terracinolide J (**1**, 2 mg). The EtOAc extracts obtained from the collections of February and March 2000, subjected to the same purification procedure, showed the presence of new compounds, along with all those previously isolated. Both extracts were chromatographed by MPLC on silica gel column (230–400 mesh) using a gradient system from hexane to EtOAc. Concerning the first one, two fractions contained terracinolides: fr. 1 (229.7 mg) eluted in hexane/EtOAc 2:8 and fr. 2 (43.4 mg) eluted in hexane/EtOAc 1:9. Fraction 1 was first separated by MPLC (from hexane/EtOAc 4:6 to EtOAc) and afforded a mixture of compounds which were separated by Si-gel HPLC (hexane/EtOAc 55:45) to give terracinolide K (**2**, 7 mg) and terracinolide C (**5**, 23 mg). Fraction 2 was purified by Si-gel HPLC (eluent hexane/EtOAc 1:1) affording 13α-OH terracinolide B (**9**, 10 mg) and 13α-OH terracinolide G (**10**, 7 mg). Finally, the extract of March 2000 showed the same diterpene composition of the extract of February 2000 but, in addition, its fr. 1 (33.3 mg), further purified by Si-gel HPLC (hexane/EtOAc 6:4), contained 13α-OH terracinolide F (**8**, 1 mg).

Terracinolide J (1). Colorless amorphous solid. $[\alpha]_D^{25}$ +11.4 (*c* 0.1 $CHCl_3$); HRFABMS (positive ions): found *m/z* 767.3488 $[M+H]^+$; calculated for $C_{38}H_{54}O_{16}$ *m/z* 767.3490; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

Terracinolide K (2). Colorless amorphous solid. $[\alpha]_D^{25}$ +26.2 (*c* 0.1 $CHCl_3$); HRFABMS (positive ion): found *m/z* 739.3239 $[M+H]^+$; calculated for $C_{36}H_{50}O_{16}$ *m/z* 739.3179; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

Terracinolide L (3). Colorless amorphous solid. $[\alpha]_D^{25}$ +6.8 (*c* 0.1 $CHCl_3$); HRFABMS (positive ion): found *m/z* 767.3399 $[M+H]^+$; calculated for $C_{38}H_{54}O_{16}$ *m/z* 767.3490; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

13α-OH Terracinolide F (8). Colorless amorphous solid. $[\alpha]_D^{25}$ +3.3 (*c* 0.1 $CHCl_3$); HRFABMS (positive ion): found *m/z* 825.3559 $[M+H]^+$; calculated for $C_{40}H_{56}O_{18}$ *m/z* 825.3545; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

Abeodendroidin F (11). Colorless amorphous solid. $[\alpha]_D^{25}$ +49.5 (*c* 0.2 $CHCl_3$); HRFABMS (positive ion): found *m/z* 701.3099 $[M+H]^+$; calculated for $C_{37}H_{48}O_{13}$ *m/z* 701.3185; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

Epiabeodendroidin F (12). Colorless amorphous solid. $[\alpha]_D^{25}$ +47.4 (*c* 0.3 $CHCl_3$); HRFABMS (positive ion): found *m/z* 701.3120 $[M+H]^+$; calculated for $C_{37}H_{48}O_{13}$ *m/z* 701.3185; ^{1}H NMR data: Table 1. ^{13}C NMR data: Table 2.

α-Ketol rearrangement of 13. To a soln of **13** (50 mg) in EtOAc (5 mL), neutral alumina (70–230 mesh, Merck, 500 mg) was added. After stirring at room temp. for 18 h, the reaction was worked up by filtration and evaporation. The solid residue was purified by CC on silica gel (1 g

silica gel, petroleum ether–EtOAc 7:3 as eluent) to afford 4 mg of recovered **13** and 30 mg (60%) of **12**, identical (^{1}H NMR, IR) to the compound obtained from the latex.

Biological assay

One million of K562/R7 human leukemic cells, expressing high levels of P-glycoprotein, were incubated for 1 h at 37 °C in 1 mL of phosphate buffer saline containing a final concentration of 10 μM daunomycin, in the presence or absence of inhibitor. The cells were then washed two times with ice-cold phosphate buffer saline, and kept on ice until analysis by flow cytometry on a FACS-II, as described previously.[6,13] Assays were performed in duplicate, in a least three separate experiments. Cyclosporin A, a potent inhibitor of P-glycoprotein was used as a positive control, at a final 2-μM concentration. The ability of 5 μM terracinolide or abeojatrophane to inhibit P-glycoprotein-mediated drug efflux was quantified by comparing the induced shift in fluorescence to that obtained with cyclosporin A.

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