Macrocyclic Diterpenoids from *Euphorbia semiperfoliata*

Giovanni Appendino,*[,†] Sven Jakupovic,[†] Gian Cesare Tron,[†] Jasmin Jakupovic,*[,‡] Veronique Milon,[‡,§] and Mauro Ballero[⊥]

Dipartimento di Scienza e Tecnologia del Farmaco, via Giuria 9, 10125 Torino, Italy, Institut für Organische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany, and Dipartimento di Scienze Botaniche, viale San Ignazio 13, 09123 Cagliari, Italy

Received November 14, 1997

In addition to known compounds, the aerial parts of *E. semiperfoliata* afforded an abietanolide (**3**), 13 jatrophane polyesters (**4**−**9**, **12**, **14**−**19**), two 4-deoxyphorbol diesters (**23**, **24**), and a pair of epimeric diterpenes (**21**, **22**) with a novel carbon skeleton, which was named euphoperfoliane. Structures were determined by spectroscopic analysis, and the main conformational features of jatropha-6(17),11-dienes are discussed in detail. The obtained isolation yield of several jatrophanes was unprecedented within the spurges (*Euphorbia* spp.), making *E. semiperfoliata* a unique source of macrocyclic diterpenoids.

Spurges (*Euphorbia* spp.) (Euphorbiaceae) are common constituents of many ancient treatments of cancer mentioned in the Greek and Roman medical literature.[1] Modern studies have also highlighted the widespread use of several of these plants to treat cancerous conditions in the traditional medicine of many areas of the world.[2] These historical and ethnobotanical clues have been backed up by the discovery of the antineoplastic activity of ingenol 3,20-dibenzoate, a constituent of the leafy spurge (*Euphorbia esula* L.).[3] However, most chemical studies on *Euphorbia* spp. have focused instead on the occurrence of skin irritant and/or tumor-promoting compounds.[4,5] Diesters of phorbol turned out to be rare and limited mainly to succulent members of the family,[4] but many highly irritant ingenol 3-monoesters have been isolated instead and shown to display tumor-promoting activity.[5] Besides the presence of ingenanes and tiglianes, spurges are of further considerable interest to natural product chemists, owing to a bewildering diversity of structurally unique macrocyclic diterpenoid constituents.[4,6] Among these, compounds of the jatrophane and lathyrane type are preeminent in terms of distribution, biogenetic relevance, and structural complexity.[4] Because these compounds are generally obtained as complex mixtures and in small isolation yield,[7] their chemical behavior is still largely unknown and their pharmacological potential presently untapped.[8]

We report here that *E. semiperfoliata* Viv. can accumulate macrocyclic diterpenoid esters in amounts unprecedented for the genus, serving as a unique source of these highly functionalized compounds. *E. semiperfoliata* is a biannual hardy spurge endemic to the mountain areas of Sardinia and Corsica, where it can form thick populations on the banks of brooks.[9] It is closely related to the wood spurge (*E. amygdaloides* L.),[10] an attractive species that finds use in homeopathic medicine.[11] The geographical isolation of Sardinia and Corsica is presumably responsible for the differentiation of these two species, which share the same chromosomal number ($2n = 20$) and can be distinguished only for morphological details in the shape of the leaves and the inflorescences.[9] Several varieties of wood spurge have been developed for gardening purposes and are commercially available in nurseries.[10] In contrast, *E. semiperfoliata* is not under cultivation,[11] and no report of previous chemical studies on this plant could be found.

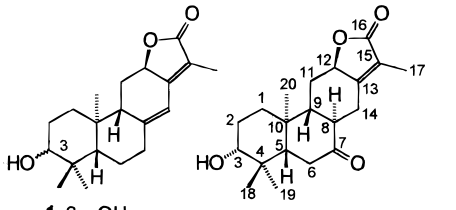
Results and Discussion

A defatted acetone extract of the aerial parts of *E. semiperfoliata* was separated using a combination of chromatographic techniques (open column chromatography on Si gel, Sephadex, and alumina; HPLC on normal- and reversed-phase columns). Twenty-five compounds were obtained. The coumarin scopoletin and abietanolides **1** and **2** (helioscopinolides A and B[12]) could be easily dereplicated. The remaining compounds were either new (**3**−**9**, **12**, **14**−**19**, **21**−**24**) or were described while this work was in progress (**10**, **11**, **13**,[13] **20**[14]).

Compound **3** had a molecular weight of 332 (HRMS), corresponding to the molecular formula $C_{20}H_{28}O_4$. The nature of the functional groups was deduced from the ^{13}C NMR spectrum, which displayed signals for a ketone carbonyl, a secondary hydroxyl, and a γ-butenolide system, the latter pointing to an abietanolide. The ^1H NMR spectrum lacked olefinic protons and thus suggested saturation of the C-8(C-14) double bond, a feature generally present in abietanolides from *Euphorbia* spp. The secondary hydroxyl was located in a C-3 equatorial orientation on the basis of the ^{13}C NMR chemical shift of ring A and the splitting pattern of the corresponding oxymethine (dd, $J = 11.0$, 4.5 Hz), which nicely fitted those reported for helioscopinolide A.[12] Thus, **3** differed from helioscopinolide A by the saturation of the C-8(C-14) double bond and the oxidation of a methylene to a ketone carbonyl. The location of the ketone carbonyl at C-7 followed from the multiplicity pattern of the protons adjacent to the carbonyl, viz., H-6 and H-8 (see Experimental Section). The detection of a large coupling between H-8 and H-9 (13.0 Hz) showed that the

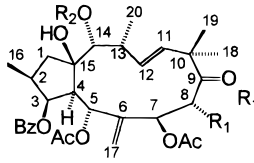
* To whom correspondence should be addressed. Tel. +39 11 670 7684 (G.A.); + 49 30 314 22564 (J.J.). Fax: + 39 11 670 7687 (G.A.); + 49 30 314 21105 (J.J.). E-mail: appendin@pharm.unito.it (G.A.); jaku@wap0107.chem-tu-berlin.de (J.J.).
† Dipartimento di Scienza e Tecnologia del Farmaco.
‡ Institut für Organische Chemie.
§ Erasmus student from the Ecole Nationale Supérieure de Chimie de Montpellier, Montpellier, France.
⊥ Dipartimento di Scienze Botaniche.

S0163-3864(97)00507-7 CCC: $15.00
Published on Web 06/04/1998

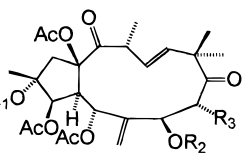


1 3α-OH
2 3β-OH

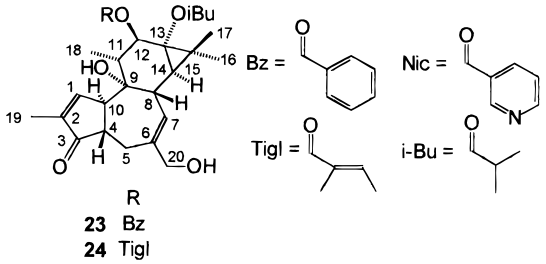
3



	R_1	R_2
4	OAc	Ac
5	OiBu	Ac
6	OH	H
7	OAc	H
8	OH	Ac
9	H	Ac



	R_1	R_2	R_3
10	Ac	Ac	OAc
11	Ac	Ac	Oi-Bu
12	Ac	Ac	OTigl
13	Ac	Ac	OBz
14	Nic	Ac	OAc
15	Ac	Bz	OAc
16	Ac	i-Bu	OAc
17	Bz	Ac	OAc
18	Ac	Ac	OH
19	Ac	i-Bu	H
20	Ac	Ac	H



23 Bz
24 Tigl

B/C rings were trans fused. Thus, **3** was assigned as 8α,14-dihydro-7-oxo-helioscopinolide A.

The ketone **4** was easy to isolate on account of its concentration (22% w/w of the purified extract, corresponding to 0.36% w/w of the dried plant material) and propensity to crystallize. HRMS established the molecular formula $C_{35}H_{44}O_{12}$, indicative of a diterpenoid polyol bearing several ester groups. The IR spectrum disclosed the presence of hydroxyl (3544 cm^{-1}) and carbonyl (1748, 1726, 1717 cm^{-1}) bands. The NMR spectra of **4** were well resolved at room temperature and amenable to extensive 2D measurements (COSY, HMBC, NOE experiments). Besides the signals of four acetates, one benzoate, four methyls, and one exocyclic methylene, three spin-systems could be identified in the ^1H NMR spectrum (Table 1). The first one (H-1α,β → H-5) started with a pair of diastereotopic methylene protons and proceeded via a methyl-coupled methine, an acyloxymethine, and a methine, to a further acyloxymethine, most probably allylic on account of its broad shape. The second spin-system (H-7,H-8) consisted of two acyloxymethines, one of which was allylic. The downfield position of the other (δ 5.74) suggested a deshielded environment, like a carbon adjacent to a carbonyl. The third sequence (H-11 → H-15) started from an olefinic proton, joined through another olefinic proton and a methyl-coupled methine to an acyloxymethine. The detection of coupling between the exomethylene protons (δ 5.14, br s and 4.96 br s, H-17a,b) and the two allylic acyloxymethines (δ 5.52, br s and 5.62 br s; H-5 and H-7, respectively) linked the first two sequences, whereas the remaining connectivity was established by inspection of the long-range ($^3J_{C-H}$) coupling pattern of the three still-unassigned nonprotonated carbons [a ketone carbonyl (δ 206.3, s, C-9), an aliphatic quaternary carbon (δ 48.3, s, C-10), and a quaternary oxygenated carbon (δ 81.4, s, C-15)]. In this way, a gross jatrophane structure was established for **4**. The next issue was the elucidation of the acylation pattern, which was solved by inspection of diagnostic $^3J_{C-H}$ couplings between oxymethine protons and carbonyl ester carbons. All ester carbonyls could be correlated to oxymethine resonances (H-3, H-5, H-7, H-8, and H-14), thus locating the free hydroxyl at the tertiary carbon. The upfield ^1H NMR resonance of the C-5 acetate (δ 1.63) is due to anisotropic shielding from the C-3 benzoate, a feature observed in all jatrophane esters of this type isolated from *E. semiperfoliata* (see Table 1, compounds **4**−**9**).

The relative configuration of **4** was assessed by analyzing the coupling constant pattern and the results of NOE experiments. In macrocyclic compounds, the proton−proton couplings are generally insufficient to define the stereochemistry, owing to the need to assume a specific conformation. However, the coupling pattern of **4** was rather peculiar, because all coupling between the aliphatic macrocyclic protons were in the 0−2 Hz range, suggesting an orthogonal relationship between them. This greatly restricted the number of possible ring conformations, and a further constraint was the presence of a trans (*E*) endocyclic double bond ($J_{11,12}$ = 16 Hz). Among the NOE effects, especially diagnostic were those of the macrocyclic protons with the methyls (H-8,H-19; H-7,H-18; H-11,H-19; H-2,H-18; H-12,H-20) and with the proton at the ring junction (H-4) (H-4,H-7; H-4,H-12), to which an α-configuration was assigned in analogy to all the other macrocyclic diterpenoids from the genus *Euphorbia*.[4,15] In a similar way, the stereochemistry at C-2 and C-3 was secured by the detection of the NOE-effects H-2,H-3 and H-3,H-4.

This general approach was then applied to the remaining jatrophanes, two of which (**5** and **9**) could also be isolated in relatively high yield (> 0.1%, see Experimental Section). The results of ^1H and ^{13}C NMR studies on these compounds are reported in Tables 1 and 2 (^1H NMR data) and 3 and 4 (^{13}C NMR data). A somewhat difficult aspect to solve was the acylation pattern of **14** and **17**, owing to the presence of two tertiary hydroxyls esterified with different acids. In these compounds, four of the five acetyls could be correlated with oxymethine protons, leaving the fifth acetate and the aromatic ester group (nicotinoate in **14**, benzoate in **17**) unassigned. This matter was settled by NOE measurements, which showed in both compounds an interaction between the aromatic protons and H-3, thus locating the aromatic group at C-2.

Overall, 13 of the jatrophane polyesters from *E. semiperfoliata* are new compounds. Compared to **4**, structural variation within these compounds stems from changes in the esterification pattern and in the oxygenation mode of the ring system. Thus, the composition of the ester moieties includes, besides acetic acid, also isobutyric, tiglic, benzoic, and nicotinic acids, with the

Table 1. ^{1}H NMR Data for the Jatrophanes **4**−**9**[a,b]

position	**4**	**5**	**6**	**7**	**8**	**9**
1α	2.20 m	2.20 m	2.39 br dd	2.38 br dd	2.19 m	2.21 m
1*β*	1.92 m	1.89 m	1.92 dd	1.92 dd	1.89 m	1.88 m
2	1.92 m	1.95 m	2.15 m	2.17 m	1.94 m	1.93 m
3	5.69 br dd	5.69 br dd	5.70 br dd	5.68 br dd	5.71 br dd	5.71 m
4	2.88 dd	2.87 dd	2.81 dd	2.84 dd	2.86 dd	2.87 dd
5	5.52 br s	5.51 br s	5.56 br s	5.46 br s	5.61 br s	5.71 m
7	5.62 br s	5.59 br s	5.42 br s	5.61 br s	5.43 br s	5.25 brd
8α						2.32 dd
8*β*	5.74 s	5.74 s	4.49 d	5.74 s	4.94 d	3.40 br d
11	6.13 d	6.13 d	5.92 d	6.07 d	5.98 d	5.98 d
12	5.89 dd	5.90 dd	6.18 dd	6.19 dd	5.88 dd	5.72 dd
13	3.20 dq	3.20 dq	3.04 dq	3.05 dq	3.18 dq	3.16 dq
14	5.13 s	5.14 s	3.70 s	3.70 s	5.13 s	5.10 s
16	0.91 d	0.91 d	0.91 d	0.91 d	0.90 d	0.91 d
17a	5.14 br s	5.11 br s	5.10 br s	5.10 br s	5.14 br s	5.04 br s
17b	4.96 br s	4.94 br s	4.91 br s	4.90 br s	4.94 br s	4.86 br s
18	1.21 s	1.19 s	1.20 s	1.21 s	1.20 s	1.21 s
19	1.23 s	1.22 s	1.23 s	1.22 s	1.23 s	1.17 s
20	1.14 d	1.14 d	1.18 d	1.17 d	1.13 d	1.13 d
OH-15	2.59 br s	2.60 br s	2.51 br s	2.54 br s	2.55 br s	2.60 br s

[a] *J*(Hz) for **4**−**8**: 1α,*β* = 13.5; 1α,2 = 6.5; 1*β*,2 = 13.0; 2,3 = 3,4 = 3.5; 2,16 = 6.5; 4.5 = 2.0; 7,8 = ca. 0; 11,12 = 16.0; 12,13 = 9.0; 13,14 = ca. 0; 13,20 = 7.0. For **9**: 2,16 = 6.5; 3,4 = 3.5; 4,5 = 2.0; 7,8α = 12,13 = 9.0; 7,8*β* = ca. 0; 8α,8*β* = 14.0; 11,12 = 16; 13,20 = 7.0; 13,14 = ca. 0. For **6** and **8**: 8, OH = 8. [b] Other signals (*δ*): For **4**: Bz 8.09 (AA′), 7.48 (BB′), 7.60 (C); OAc-5 1.63 (s); OAc-7 2.12 (s); OAc-8 2.07 (s); OAc-14 2.21 (s). For **5**: Bz 8.09 (AA′), 7.48 (BB′), 7.60 (C); OAc-5 1.64 (s); OAc-7 2.07 (s); 8-^{i}Bu: 2.63 (m), 1.18 (d, *J* = 7.0 Hz); 1.16 (d, *J* = 7.0 Hz); OAc-14 2.23 (s). For **6**: Bz 8.11 (AA′), 7.48 (BB′), 7.60 (C); OAc-5: 1.69 (s); OAc-7: 2.07 (s); OH-8 3.21 (d). For **7**: Bz 8.10 (AA′), 7.47 (BB′), 7.59 (C); OAc-5 1.64 (s); OAc-7 2.11 (s); OAc-8: 2.06 (s). For **8**: Bz 8.09 (AA′), 7.49 (BB′), 7.60 (C); OAc-5 1.66 (s); OAc-7 2.08 (s); OH-8 3.20 (d); OAc-14 2.20 (s). For **9**: Bz 8.09 (AA′), 7.48 (BB′), 7.60 (C); OAc-5 1.62 (s); OAc-7 2.11 (s); OAc-14 2.19 (s).

Table 2. ^{1}H NMR Data for the Jatrophanes 12 and **14**−**19**[a,b]

position	**12**	**14**	**15**	**16**	**17**	**18**	**19**
1α	3.88 dd	4.05 dd	3.92 dd	3.86 dd	4.08 dd	3.88 dd	3.87 dd
1*β*	1.92 d	2.06 d	1.96 d	1.93 d	2.05 d	1.92 d	1.92 d
3	5.51 dd	5.74 dd	5.55 dd	5.52 dd	5.74 dd	5.53 dd	5.54 dd
4	2.92 dd	2.92 dd	3.10 dd	2.94 dd	2.96 dd	2.91 dd	2.83 dd
5	5.75 br s	5.78 br s	5.80 br s	5.70 br s	5.70 br s	5.81 br s	5.85 br s
7	5.41 br s	5.34 br s	5.64 br s	5.41 br s	5.41 br s	5.20 br s	4.97 br s
8α							2.78 dd
8*β*	5.63 s	5.65 s	5.74 s	5.62 s	5.69 s	4.77 s	3.23 brd
11	6.26 d	6.22 d	6.29 d	6.22 d	6.22 d	6.09 d	6.08 d
12	5.60 dd	5.41 dd	5.66 dd	5.61 dd	5.46 dd	5.59 dd	5.49 dd
13	3.64 dq	3.61 dq	3.68 dq	3.63 dq	3.62 dq	3.63 dq	3.57 dq
16	1.50 s	1.65 s	1.51 s	1.50 s	1.65 s	1.51 s	1.47 s
17a	5.26 br s	5.25 br s	5.29 br s	5.26 br s	5.28 br s	5.27 br s	5.13 br s
17b	5.24 br s	5.23 br s	5.29 br s	5.21 br s	5.27 br s	5.23 br s	5.10 br s
18	1.16 s	1.10 s	1.14 s	1.13 s	1.12 s	1.19 s	1.17 s
19	1.27 s	1.21 s	1.25 s	1.23 s	1.22 s	1.22 s	1.17 s
20	1.15 d	1.08 d	1.18 d	1.15 d	1.08 d	1.16 d	1.18 d

[a] *J*(Hz). For **12**, **14**−**19**: 1α,1*β* = 11,12 = 16.0; 1α,3 = 4, 5 = 1.0; 3,4 = 4.0; 7,8 = ca. 0; 12,13 = 9.0; 13,20 = 7.0. For **19**: 7,8α = 9.0; 8α,8*β* = 14.0. For **18**: 8,OH = 8.5. [b] Other signals. For **12**: 5 × OAc 2.16 (s), 2.11 (s), 2.09 (s), 2.08 (s), 2.05 (s); OTigl 6.92 (br q, *J* = 7.0); 1.79 (br d, *J* = 7.0); 1.82 (br s). For **14**: 5 × OAc 2.21 (s), 2.14 (s), 2.14 (s), 2.11 (s), 2.07 (s); ONic 9.27 (br s), 8.74 (dd, *J* = 5.0, 2.0 Hz), 8.38 (ddd, *J* = 8.0, 2.0, 2.0), 7.35 (br dd, *J* = 5.0, 2.0). For **15**: 5 × OAc 2.21 (s), 2.16 (s), 2.16 (s), 2.14 (s), 2.12 (s); Bz 7.99 (AA′), 7.44 (BB′), 7.58 (C). For **16**: 5 × OAc 2.16 (s), 2.12 (s), 2.10 (s), 2.10 (s), 2.09 (s); OiBu 2.55 (m), 1.10 (d, *J* = 7.0), 1.08 (d, *J* = 7.0). For **17**: 5 × OAc 2.21 (s), 2.14 (s), 2.13 (s), 2.11 (s), 2.07 (s); OBz 8.01 (AA′), 7.40 (BB′), 7.52 (C). For **18**: 5 × OAc 2.18 (s), 2.13 (s), 2.12 (s), 2.09 (s), 2.01 (s); OH-8 3.35 (d). For **19**: 4 × OAc 2.16 (s), 2.14 (s), 2.12 (s), 2.03 (s); OiBu 2.59 (m), 1.13 (d, *J* = 7.0), 1.12 (d, *J* = 7.0).

aromatic acid moiety(moieties) present at C-2, C-3, C-7, and C-8, but not at C-5 and C-14. Compared to **4**, a further oxygen function can be found at C-2 (acyloxy group), whereas the acyloxy group at C-14 can be replaced by a keto group, and C-8 was not oxygenated in three compounds (**9**, **19**, **20**). Interestingly, all compounds oxygenated at C-2 had the tertiary C-15 hydroxyl acetylated, and a keto group at C-14, whereas an acetoxyl at C-5 and a carbonyl at C-9 were present in all macrocyclic polyesters. The compounds nonoxygenated at C-2 had a benzoyloxy group at C-3 (**4**−**9**), replaced by an acetoxyl in compounds bearing a C-2 oxygen function (**10**−**20**). All compounds with a keto group at C-14 were fully acylated, with the exception of **18**, having a free hydroxyl at C-8; those with a tetrahedral C-14 had instead one or more (up to three) free hydroxyls. In all cases, the stereochemistry at the stereogenic centers was always identical, as suggested by the same coupling-constant pattern, and confirmed by NOE measurements. The jatrophane polyesters from *E. semiperfoliata* can thus be divided in two groups, one based on a 3,5,7,(8),14,15-hexa(penta)hydroxy-9-oxojatropha-6(17),11*E*-diene parent structure (compounds **4**−**9**) and the other on a 2,3,5,7,(8),15-hexa(penta)-hydroxy-9,14-dioxojatropha-6(17),11*E*-diene model (compounds **10**−**20**).

Notwithstanding an identical configuration at the stereogenic centers, jatropha-6(17),11-dienes can adopt different conformations, depending mainly on their esterification pattern.[16] In compounds with a 13α methyl group, these differences are related to a different orientation of the exomethylene in regards to the mean

Table 3. ^{13}C NMR Data for the Jatrophanes **4**, **5**, and **7**−**10**[a]

carbon	4	5	7	8	9	10[b]
1	50.3 t	50.3 t	51.5 t	50.3 t	50.4 t	46.1 t
2	36.9 d	36.9 d	36.8 d	37.0 d	36.9 d	86.1 s
3	78.8 d	78.8 d	79.7 d	78.9 d	79.2 d	78.0 d
4	46.5 d	46.7 d	45.1 d	46.5 d	46.4 d	49.3 d
5	71.0 d	71.1 d	71.4 d	71.0 d	71.0 d	67.9 d
6	143.5 s	143.4 s	143.5 s	144.1 s	147.8 s	143.0 s
7	67.8 d	67.3 d	68.1 d	69.1 d	69.7 d	67.7 d
8	74.7 d	74.9 d	74.9 d	73.8 d	45.7 t	74.2 d
9	206.3 s	206.2 s	206.8 s	212.3 s	210.8 s	205.5 s
10	48.3 s	48.2 s	48.4 s	47.8 s	49.4 s	48.4 s
11	135.7 d	135.9 d	134.9 d	135.2 d	136.7 d	136.0 d
12	132.5 d	132.4 d	133.1 d	133.0 d	132.3 d	133.4 d
13	36.1 d	36.2 d	36.6 d	36.2 d s	36.2 d	44.6 d
14	80.8 d	80.8 d	81.1 d	80.8 d	80.6 d	210.5 s
15	81.4 s	81.6 s	82.5 s	81.6 s	81.9 s	91.4 s
16	13.7 q	13.7 q	13.7 q	13.7 q	13.7 q	17.6 q
17	111.0 t	111.0 t	110.7 t	110.7 t	110.0 t	113.0 t
18	28.8 q	22.8 q	23.2 q	22.8 q	22.8 q	22.5 q
19	27.6 q	27.6 q	27.7 q	27.4 q	27.6 q	27.6 q
20	24.9 q	25.0 q	25.3 q	24.8 q	25.0 q	20.2 q

[a] Other signals. For **4**: 5-OAc 169.7 (s), 20.1 (q); 7-OAc 169.5 (s), 20.7 (q); 8-OAc 170.1 (s), 20.7 (q); 14-OAc 170.5 (s), 20.8 (q); 3-OBz 165.3 (s), 130.2 (s), 129.4 (d), 128.5 (d), 133.2 (d). For **5**: 5-OAc 169.8 (s), 20.2 (q); 7-OAc 169.5 (s), 20.7 (q); 14-OAc 170.6 (s), 20.9 (q); 3-OBz 165.3 (s), 130.3 (s), 129.5 (d), 128.5 (d), 133.2 (d), 8-OiBu 176.0 (s), 33.8 (d), 19.0 (q), 18.8 (q). For **7**: 5-OAc 169.8 (s), 20.2 (q); 7-OAc 169.6 (s), 20.7 (q); 3-OBz 165.4 (s), 130.4 (s), 129.5 (d), 128.5 (d), 133.2 (d). For **8**: 5-OAc170.0 (s), 20.8 (q); 7-OAc 170.0 (s), 20.7 (q); 14-OAc 170.5 (s), 20.8 (q); 3-OBz 165.3 (s), 130.3 (s), 129.5 (d), 128.6 (d), 133.2 (d). For **9**: 5-OAc 170.3 (s), 20.7 (q); 7-OAc 170.0 (s), 20.8 (q); 14-OAc 170.6 (s), 20.9 (q); 3-OBz 165.2 (s), 130.4 (s), 129.4 (d), 128.6 (d), 133.2 (d). For **10**: 6 × OAc 170.3 (s), 170.1 (s), 169.8 (s), 169.4 (s), 169.3 (s), 169.1 (s), 22.0 (q), 21.3 (q), 21.0 (q), 20.7 (q), 20.5 (q), 20.2 (q). [b] Data not available in Jakupovic et al.

Table 4. ^{13}C NMR Data for the Jatrophanes **12**, **14**, **16**, and **19**[a]

carbon	12	14	16	19
1	46.2 t	46.4 t	46.4 t	46.5 t
2	86.2 s	87.9 s	86.0 s	86.0 s
3	78.1 d	77.9 d	77.9 d	78.3 d
4	49.4 d	49.4 d	49.4 d	49.5 d
5	68.0 d	67.9 d	68.1 d	68.0 d
6	143.4 s	142.8 s	143.4 s	146.7 s
7	68.0 d	68.0 d	67.4 d	69.3 d
8	74.3 d	74.2 d	74.5 d	45.5 t
9	205.5 s	205.4 s	205.3 s	209.6 s
10	48.5 s	48.4 s	48.4 s	48.3 s
11	136.3 d	136.5 d	136.0 d	137.2 d
12	133.3 d	133.0 d	133.5 d	132.9 d
13	44.8 d	44.7 d	44.7 d	44.5 d
14	210.6 s	210.0 s	210.5 s	210.1 s
15	91.6 s	91.4 s	91.5 s	92.1 s
16	17.6 q	17.9 q	17.6 q	17.7 q
17	113.1 t	113.2 t	112.9 t	110.3 t
18	22.6 q	22.6 q	22.6 q	22.8 q
19	27.9 q	27.6 q	27.7 q	26.7 q
20	20.3 q	20.2 q	20.3 q	20.2 q

[a] Other signals. For **12**: 5 × OAc 170.4 (s), 170.1 (s), 169.5 (s), 169.2 (s), 169.2 (s), 22.1 (q), 21.4 (q), 21.1 (q), 20.8 (q), 20.6 (q); 8-OTigl 166.0 (s), 138.6 (s), 127.7 (d), 12.0 (q), 14.5 (q). For **14**: 5 × OAc 170.1 (s), 169.8 (s), 169.8 (s), 169.3 (s), 169.1 (s), 21.3 (q), 21.1 (q), 20.8 (q), 20.6 (q), 20.1 (q), 2-ONic 164.3 (s), 151.2 (d), 125.9 (s), 137.5 (d), 123.1 (d), 152.9 (d). For **16**: 5 × OAc 170.4 (s), 170.1 (s), 169.9 (s), 169.3 (s), 169.1 (s), 22.0 (q), 21.3 (q), 21.1 (q), 20.8 (q), 20.3 (q); 7-OiBu 175.6 (s), 33.5 (d), 18.9 (q), 18.6 (q). For **19**: 4 × OAc 170.1 (s), 169.7 (s), 169,3 (s), 169.0 (s), 21.3 (q), 21.2 (q), 20.9 (q), 20.7 (q); 7-OiBu 175.3 (s), 34.6 (d), 18.7 (d), 18.6 (q).

plane of the macrocycle. In one type, the exomethylene is in the mean plane of the macrocycle ("exo" conformation, Figure 1A), H-4 and H-5 are almost orthogonal, and H-5 is close to H-8β.[16] Diagnostic NMR features are a small value of $J_{4,5}$ (0−3 Hz) and the detection of H-4/H-5 and H-5/H-8β NOE effects. In the other conformation, the exomethylene is above the mean plane of the macrocycle ("endo" conformation, Figure 1B), H-4 and H-5 are antiperiplanar, and H-5 is close to the exomethylene, as shown by a large value of $J_{4,5}$ (9−11 Hz) and by the detection of a NOE effect between H-5 and one of the methylene protons (H-17a).[16] The small value of $J_{4,5}$ (1−2 Hz) and the detection of H-4/H-5 and H-5/H-8β NOE effects showed that all jatrophadienes from *E. semiperfoliata* belong to the same "exo" conformational type.

In topological terms, the 12-membered ring of jatrophadienes is reminiscent of the 10-membered ring of germacradienes. It might thus be convenient to extend certain conventions proposed for medium-sized sesquiterpenoids[17] to medium-sized diterpenoids. The orientation of the groups on the double bonds below (α), in, or above (β) the mean plane of the cycle might thus be indicated by an index, a prefix or a suffix next to the capital D. According to these conventions, orienting the molecule with the five-membered ring on the left side of the observer and with a counterclockwise numbering sequence from C-1, the conformation of jatrophadienes **1**−**17** would be of the 17D, $_{11}$D^{12} -type, whereas the other conformation would be of the ^{17}D, $_{11}$D^{12}-type. The use of descriptors typical of six-membered rings (chair, boat, twist, etc.) is somewhat ambiguous in medium-sized rings, and the ^{x}D^{y} convention complements that based on the "endo", "exo" descriptors,[16] being also applicable to jatrophane rotamers differing in the orientation of the endocyclic double bond(s).

Jatrophanes of the $\Delta^{6(17)}$, Δ^{11} type have so far been considered less common than those having two endocyclic double bonds,[4,7] but the set of new compounds isolated from *E. semiperfoliata* in the present study expands considerably the database for these compounds.[4b,c]

In addition to 12-membered jatrophane derivatives, two 11-membered macrocyclic diterpenoids of a novel structural type were also isolated (**21** and **22**). These compounds were obtained and characterized as a 5:2 mixture, though an analytical sample of the major epimer was later obtained by reversed-phase HPLC. The presence of the same proton spin-systems suggested that **21** and **22** are stereoisomers. Their ^{1}H NMR spectra (Table 5) consisted of five spin systems, three of which closely matched those of the jatrophanes **10**−**20** (AB system for H-2α,β; AMX system for H-3, H-4, and H-5; ABX system for H-11, H-12, and H-13). The remaining signals were a broadened olefinic methine singlet and an AB aliphatic methylene quartet, which replaced the signals of the exomethylene and of H-7 and H-8 in **10**−**20**. Based on these data, a jatrophane-like connectivity modified in the C-6−C-9 moiety could be envisioned for **21** and **22**. The ^{13}C NMR spectrum of **21** confirmed this, showing that the functionality of the C-6−C-9 moiety comprised a trisubstituted double bond [δ 174.7 (s) and 131.2 (d)], a quaternary oxygenated carbon (δ 81.8, s), and an aliphatic methylene (δ 42.3, t). Among the possible combinations of these structural elements, a conjugated cyclopentenone seemed likely on the basis of the chemical shift of the ketone carbonyl and the olefinic double bond.[18] This was confirmed by

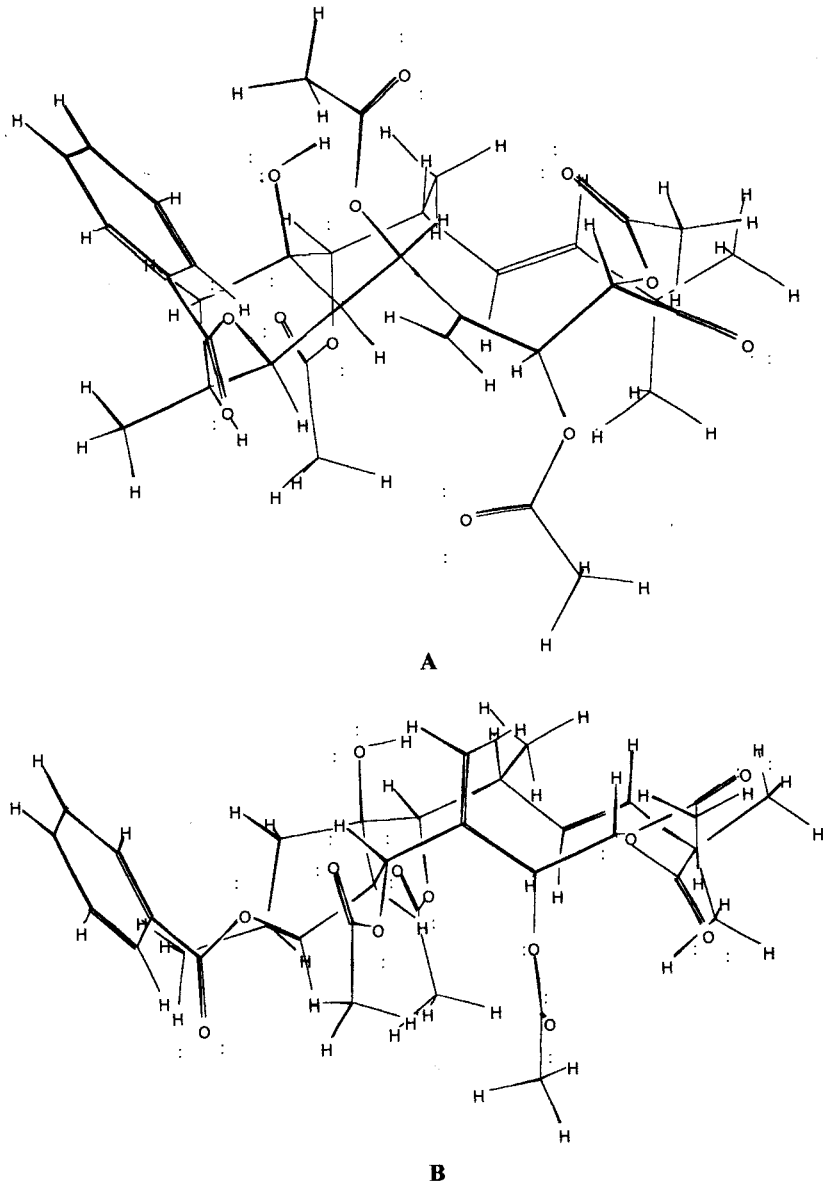


Figure 1. Major conformational isomers of jatrophanes of the $\Delta^{6(17),11}$-diene type, as typified by **4**.

the detection of diagnostic HMBC correlations. Especially relevant were the ^3J correlations H-17a/C-8, H-17b/C-7, and H-7/C-9, whereas the site of attachment to the rest of the diterpenoid core was indicated by the correlations H-18/C-9 and H-19/C-9 on one side, and H-5/C-7 on the other. The detection of NOE effects between H-7 and H-4 in **21**, and between H-7 and H-5 in **22**, showed that these compounds differ for the orientation of the C-6, C-9 methylene bridge, which is "β" in **21** (α-9-hydroxyl) and "α" in **22** (β 9-hydroxyl) (Figure 3). The topology of **21** and **22** is unprecedented within the diterpenoids, and we have named their skeleton euphoperfoliane. From a biogenetic point of view, the euphoperfoliane skeleton might derive from a jatrophane precursor of the Δ,[6] C-8,C-9-dione-type via an intramolecular vinylogous aldol condensation (Figure 2). A reaction of this type is also involved in the epimerization of phorbol to 4α-phorbol.[19]

The two remaining diterpenoid esters were C-12,C-13-diesters of 4-deoxyphorbol,[20] both bearing an isobutyrate and differing only in the nature of the other ester group (benzoate in **23**, tiglate in **24**). The ^{1}H NMR data (see Experimental Section) for the diterpenoid core matched those for 4-deoxyphorbol,[4] and the esterification pattern was established by inspection of the NOE effects. In both compounds, NOE-correlations between the isobutyrate α-protons and H-16 and H-14 could be detected, thus locating the saturated ester at C-13. This was further confirmed by the observation of NOE effects between the benzoate (tiglate) protons and H-11. 4-Deoxyphorbol esters are rare in herbaceous *Euphorbia* species, but occur more frequently in the cactaceous species from the subsection *Euphorbium*.[4]

From a taxonomic stand point, *E. semiperfoliata* is placed in the subsection *esulae* Boiss.,[21] but the absence of ingenane derivatives and the occurrence of jatrophanes and abietanolides are rather unusual for spurges of this section, though the lack of ingenol derivatives has also been reported in the wood spurge.[11]

The diterpenoids from *E. semiperfoliata* add to the burgeoning array of unique isoprenoids isolated from Sardinian plants[22] and should help to provide an incentive for biodiversity conservation and further chemical prospecting in those areas of the Mediterranean regions

Table 5. ^{1}H NMR Data for the Euphoperfolinanes **21**, **22**;[a,b] and ^{13}C NMR Data for the Euphoperfoliane **21**[c]

position	**21**	**22**	carbon	**21**
1α	3.33 dd	3.73 dd	1	44.9 t
1β	2.58 d	2.00 d	2	86.3 s
3	5.44 dd	5.41 dd	3	78.7 d
4	3.86 dd	3.09 dd	4	54.4 d
5	5.29 d	6.12 d	5	66.8 d
7	6.23 dd	6.32 dd	6	174.7 s
11	5.00 d	5.07 d	7	131.2 d
12	5.48 dd	5.01 dd	8	209.3 s
13	3.57 dq	3.12 dq	9	81.8 s
16	1.44 s	1.46 s	10	43.4 s
17a	2.87 dd	2.54 dd	11	139.0 d
17b	2.79 dd	2.37 dd	12	123.7 d
18	0.99 s	1.03 s	13	42.9 d
19	1.11 s	1.09	14	206.6 s
20	1.07 d	1.07 d	15	93.0 s
			16	18.3 q
			17	42.3 t
			18	20.5 q
			19	20.1 q
			20	16.8 q

[a] *J* (Hz) for **21** and **22**: 1α,1β = 16.0; 1α,3 = 2.0; 3,4 = 4; 4,5 = 11.0; 7,17a = 2.0; 7,17b = 2.5; 11,12 = 16.0; 12,13 = 9.0; 13,20 = 7.0; 17a,17b = 18.0. [b] Other proton signals. For **21**: OH-9 2.61 (br s); 4 × OAc 2.31 (s), 2.11 (s), 2.06 (s), 2.00 (s). For **22**: OH-9 2.65 br s; 4 × OAc 2.22 (s), 2.21 (s), 2.08 (s), 1.98 (s). [c] Other carbon signals for **21**: 4 × OAc 170.2 (s), 169.8 (s), 169.1 (s), 168.8 (s), 22.2 (q), 22.0 (q), 20.6 (q), 20.6 (q).

still rich in biodiversity. In this context, Sardinia is a striking example, because about 10% of its native vascular plants are endemic (overall 202 species, belonging to 114 genera, one of which is unique).[23] Furthermore, the presence of unusual chemotypes has often been detected in several non-endemic species collected in this island,[24] making its flora a valuable storehouse of chemical diversity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Perkin−Elmer model 237 spectrophotometer. HRMS were obtained on a MAT 95ST Finnigan MAT apparatus (70 eV, EI mode). ^{1}H and ^{13}C NMR spectra were taken on a Bruker AM 400 spectrometer (400 and 100 MHz, respectively). ^{1}H and ^{13}C NMR chemical shifts refer to C*H*Cl$_3$ at 7.26 ppm, and *C*DCl$_3$ at 77.0 ppm, respectively. Si gel 60 (70−230 mesh, Merck) was used for open column chromatography. A Waters Microporasil column (0.8 × 30 cm) was used for HPLC and a LiChrosorb C$_{18}$ column (0.8 × 25 cm) for reversed-phase HPLC, with detection by a Waters differential refractometer 340. Figures 1 and 3 were generated with PCMODEL, Serena Software, Vers. 4.0.

Plant Material. Aerial parts of *E. semiperfoliata* Viv. were collected around Arzana (Nuoro) in October 1996, and May 1997. The plant material was identified by M. B., and a voucher specimen (1217) is kept at the Dipartimento di Scienze Botaniche, University of Cagliari.

Extraction and Isolation. Dried and powdered plant material (350 g) was extracted with Me$_2$CO at room temperature (1 × 1.5 L, 2 × 1 L). The pooled extracts were evaporated in vacuo, and the residue was suspended in EtOH (350 mL) and treated with an equal volume of 3% Pb(OAc)$_2$. After about 5 h the suspension was filtered on a bed of Celite, and the clear filtrate was concentrated in vacuo to remove most of the EtOH and then extracted with EtOAc. After washing with brine, drying (Na$_2$SO$_4$), and evaporation, 5.58 g of residue were obtained. The latter was purifed by open column chromatography on Si gel (ca. 40 g), using mixtures of hexane and EtOAc (from 9:1 to 3:7). According to differences in composition indicated by TLC, seven crude fractions were obtained. Fractions A and B contained triterpenoids and fats and were not further investigated. Fractions C, D, and E crystallized from ether, affording 728 mg **9** (0.21%), 434 mg **5** (0.12%), and 1.160 g **4**, respectively. Fraction F was further separated by column chromatography (hexane−EtOAc 1:1) to give a further 100 mg of **4** (overall yield: 1.260 g, 0.36%), and two mixtures, which were further separated by HPLC on Si gel (hexane−EtOAc 1:1), to give **8** (61 mg), **11** (12 mg), **19** (23 mg) and **7** (16 mg), **17** (6 mg), **20** (12 mg), and **16** (11 mg), respectively. Fraction G was separated by column chromatography (hexane−EtOAc 5:5) to give **10** (45 mg) and a mixture, which was further separated by HPLC on Si gel (hexane−EtOAc 2:3), affording **20** (11 mg), **1** (8 mg), **2** (13 mg), **3** (4 mg), **7** (21 mg), **12** (18 mg), and **13** (7 mg). Column chromatography of the most polar fraction (H) on Sephadex LH-20 using hexane−EtOAc 4:6 as eluent afforded the coumarin scopoletin (19 mg) and a mixture, which was further purified by column chromatography on neutral alumina (eluent hexane−EtOAc 6:4) to afford **14** (57 mg) and a mixture of several compounds. The latter was purified by HPLC on a RP-18 column (H$_2$O−MeOH 3:2) to give **6** (3 mg), a 5:2 mixture of **21** and **22** (7 mg), **17** (3 mg), **23** (3 mg), and **24** (3 mg).

8α,14-Dihydro-7-oxohelioscopinolide A (3): white powder, mp 138−141 °C; $[\alpha]^{25}_D$ −21° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3551, 1738, 1705, 1684, 1418, 1231, 1094, 1036, 1009 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.84 (1H, br t, *J* = 8.0 Hz, H-12), 3.24 (1H, dd, *J* = 11.0, 4.5 Hz, H-3), 2.82 (2H, br d, *J* = 8.0 Hz, H-14a,b), 2.61 (1H, dt, *J* = 12.0, 8.0 Hz, H-8), 2.50 (1H, dd, *J* = 14.0, 3.5 Hz, H-6β), 2.43 (1H, t, *J* = 14.0 Hz, H-6α), 2.29 (1H, m, H-11a), 1.80 (3H, br s, H-17), 1.51 (1H, m, H-11b), 1.37 (1H, m, H-9), 1.13 (3H, s, H-18), 0.97 (3H, s, H-20), 0.87 (3H, s, H-19); ^{13}C NMR (CDCl$_3$) δ 28.2 (t, C-1), 26.8 (t, C-2), 78.1 (d, C-3), 37.2 (s, C-4), 43.8 (d, C-5), 35.7 (t, C-6), 209.4 (s, C-7), 49.4 (d, C-8), 53.02 (d, C-9), 39.1 (s., C-10), 23.8 (t, C-11), 77.0 (d, C-12), 160.4 (s, C-13), 38.5 (t, C-14), 122.0 (s, C-15), 174.8 (s, C-16), 8.4 (q, C-17), 27.6 (q, C-18), 14.9 (q, C-19), 13.1 (q, C-20); MS *m/z* 332.199 [M]$^+$(0.5) (calcd for C$_{20}$H$_{28}$O$_4$, 332.199), 310 [M − H$_2$O] (5), 286 (40), 169 (80), 106 (100).

(2*S,3*S**,4*R**,5*R**,7*S**,8*R**,13*R**,14*R**,15*R**)-5,7,8,-14-Tetraacetoxy-3-benzoyloxy-15-hydroxy-9-oxojatropha-6(17),11*E*-diene (4):** white powder: mp 220−221 °C; $[\alpha]^{25}_D$ −210° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3544, 1748, 1726, 1717, 1373, 1277, 1225, 1071, 716 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MS *m/z* 656.283 [M]$^+$ (0.5) (calcd for C$_{35}$H$_{44}$O$_{12}$, 656.283), 638 [M − H$_2$O]$^+$ (2), 596 [M − AcOH]$^+$ (7), 105 [PhCO] (100).

(2*S,3*S**,4*R**,5*R**,7*S**,8*R**,13*R**,14*R**,15*R**)-5,7,14-Triacetoxy-3-benzoyloxy-8-isobutyroyloxy-15-hydroxy-9-oxojatropha-6(17),11*E*-diene (5):** white powder; mp 172−174 °C; $[\alpha]^{25}_D$ +140 ° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3579, 3470, 1742, 1728, 1716, 1383, 1277,

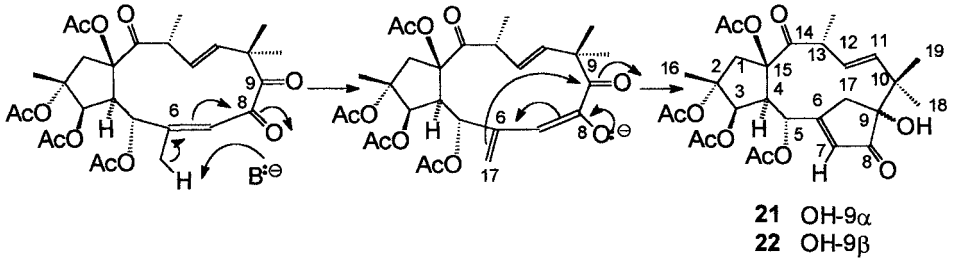


Figure 2. Possible biogenetic origin of the euphoperfolianes **21** and **22** from a jatrophane precursor

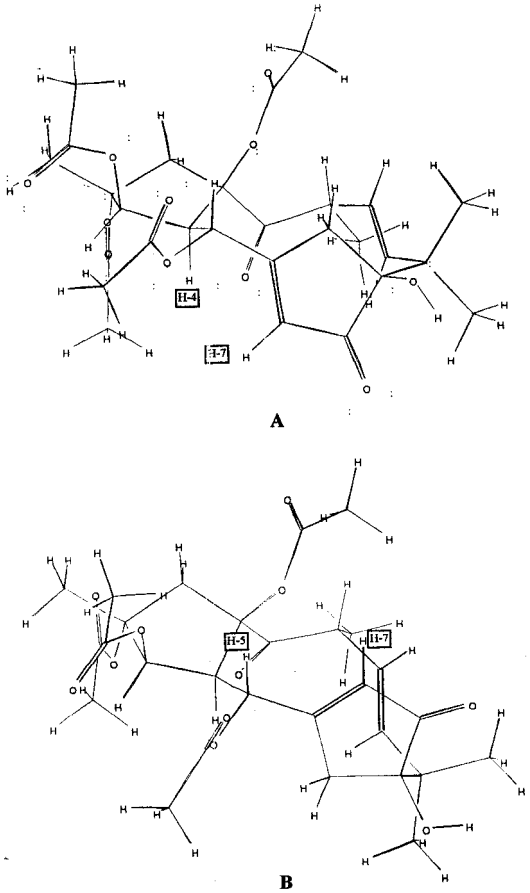


Figure 3. Conformation of the euphoperfolianes **21** (**A**) and **22** (**B**) (PCModel).

1288, 1030, 717 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MS *m/z* 684.315 $[M]^+$ (0.5) (calcd for $C_{37}H_{48}O_{12}$, 684.315), 66 $[M - H_2O]^+$ (2), 624 [M − AcOH] (4), 596 [M − *i*BuOH] (5), 105 [PhCO] (100).

(2*S,3*S**,4*R**,5*R**,7*S**,8*R**,13*R**,14*R**,15*R**)-5,7-Diacetoxy-3-benzoyloxy-8,14,15-trihydroxy-9-oxojatropha-6(17),11*E*-diene (6):** gum; $[\alpha]^{25}_D$ −130° (*c* 0.08, MeOH); IR (liquid film) ν_{max} 3700, 3370, 1750, 1720, 1700, 1380, 1267, 1248, 1090, 700 cm^{-1}; ^1H NMR data, see Table 1; MS *m/z* 554.251 $[M - H_2O]^+$ (0.1) (calcd for $C_{31}H_{38}O_9$ 554.251), 512 $[M - AcOH]^+$ (0.2), 494 [554 − AcOH]$^+$ (0.6), 452 [512 − AcOH]$^+$ (5), 177 (10), 105 $[PhCO]^+$ (100).

(2*S,3*S**,4*R**,5*R**,7*S**,8*R**,13*R**,14*R**,15*R**)-5,7,8-Triacetoxy-3-benzoyloxy-14,15-dihydroxy-9-oxojatropha-6(17),11*E*-diene (7):** white powder; mp 146−150 °C; $[\alpha]^{25}_D$ −315° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3560, 1748, 1717, 1699, 1375, 1281, 1227, 1032, 719 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MS *m/z* 614.273 $[M]^+$ (0.5) (calcd for $C_{33}H_{42}O_{11}$, 614.273), 596 $[M - H_2O]$ (2), 554 [M − AcOH] (2), 384 (10), 266 (16), 105 (100) [PhCO].

(2*S,3*S**,4*R**,5*R**,7*S**,8*R**,13*R**,14*R**,15*R**)-5,7,14-Triacetoxy-3-benzoyloxy-8,15-dihydroxy-9-oxojatropha-6(17),11*E*-diene (8):** white powder; mp 105−107 °C; $[\alpha]^{25}_D$ −108° (*c* 0.15, MeOH); IR (KBr) ν_{max} 3488, 1744, 1719, 1373, 1279, 1229, 1030, 713 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MS *m/z* 614.273 $[M]^+$ (0.5) (calcd for $C_{33}H_{42}O_{11}$, 614.273), 596 $[M - H_2O]$ (2), 554 [M − AcOH] (3), 384 (10), 266 (16), 105 (100) [PhCO].

(2*S,3*S**,4*R**,5*R**,7*R**,13*R**,14*R**,15*R**)-5,7,14-Triacetoxy-3-benzoyloxy-15-hydroxy-9-oxojatropha-6(17),11*E*-diene (9):** white powder: mp 175−177 °C; $[\alpha]^{25}_D$ −160° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3524, 1746, 1725, 1698, 1371, 1279, 1229, 1030, 716 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MS *m/z* 598.278 $[M]^+$ (0.1) (calcd for $C_{33}H_{42}O_{10}$, 598.278), 580 $[M - H_2O]$ (2), 520 $[M - H_2O - AcOH]$ (2), 105 (100) [PhCO].

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-2,3,5,7,15-Pentaacetoxy-8-tigloyloxy-9,14-dioxojatropha-6(17),-11*E*-diene (12):** white powder, mp 108 °C; $[\alpha]^{25}_D$ − 120° (*c* 0.10, MeOH); IR (KBr) ν_{max}1741, 1720, 1700, 1371, 1279, 1229, 1030, 720 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; MS *m/z* 690.289 $[M]^+$ (4) (calcd for $C_{35}H_{46}O_{14}$, 690.289), 96 (95), 83 $[C_4H_7CO]^+$ (100).

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-3,5,7,8,15-Pentaacetoxy-2-nicotinoyloxy-9,14-dioxojatropha-6(17),11*E*-diene (14):** gum; IR (liquid film) ν_{max}1740, 1720, 1706,1700, 1380, 1280, 1235, 1050, 730 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; MS *m/z* 713.268 $[M]^+$ (0.2) (calcd for $C_{36}H_{43}NO_{14}$, 713.268), 653 [M − AcOH] (1), 506 (2), 106 (100) [ArCO].

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-2,3,5,8,15-Pentaacetoxy-7-benzoyloxy-9,14-dioxojatropha-6(17),11*E*-diene (15):** gum; $[\alpha]^{25}_D$ −65° (*c* 0.10, MeOH); IR (liquid film) ν_{max} 1740, 1710, 1695, 1335, 1262, 1245, 1130, 740 cm^{-1};^1H NMR data, see Table 2; MS *m/z* 712.272 $[M]^+$ (1) (calcd for $C_{37}H_{44}O_{14}$,712.273), 123 (16), 105 $[PhCO]^+$ (100).

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-2,3,5,8,15-Pentaacetoxy-7-isobutyroyloxy-9,14-dioxojatropha-6(17),11*E*-diene (16):** gum; $[\alpha]^{25}_D$ −81° (*c* 0.10, MeOH); IR (liquid film) ν_{max} 1738, 1718, 1703, 1375, 1282, 1225, 1130, 740 cm^{-1};^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4, MS *m/z* 678.289 $[M]^+$ (0.4) (calcd for $C_{34}H_{46}O_{14}$, 678.289), 618 [M − AcOH] (5), 506, (20), 83 (100).

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-3,5,7,8,15-Pentaacetoxy-2-benzoyloxy-9,14-dioxojatropha-6(17),11*E*-diene (17):** gum; IR (liquid film) ν_{max} 1743,

1721, 1700, 1382, 1290, 1222, 1090, 740 cm^{-1}; ^1H NMR data, see Table 2; MS *m/z* 712.273 $[M]^+$ (5) (calcd for $C_{37}H_{44}O_{14}$, 712.273), 123 (30), 105 $[PhCO]^+$ (100).

(2*R,3*R**,4*S**,5*R**,7*S**,8*R**,13*R**,15*R**)-2,3,5,7,15-Pentaacetoxy-8-hydroxy-9,14-dioxojatropha-6(17),-11*E*-diene (18):** gum; $[\alpha]^{25}_D$ −45° (*c* 0.10, MeOH); IR (liquid film) ν_{max}1740, 1725, 1700, 1312, 1270, 1229, 1099 cm^{-1};^1H NMR data, see Table 2; MS *m/z* 608.246 $[M]^+$ (6) (calcd for $C_{30}H_{40}O_{13}$ 608.247), 566 [M − ketene]$^+$ (4), 548 [M − AcOH]$^+$ (8), 506 [566 − AcOH]$^+$ (18), 296 (85), 202 (62), 96 (100).

(2*R,3*R**,4*S**,5*R**,7*R**,13*R**,15*R**)-2,3,5,15-Tetraacetoxy-7-isobutyroyloxy-9,14-dioxojatropha-6(17),-11*E*-diene (19):** white powder; mp 143−145 °C; $[\alpha]^{25}_D$ −90° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3542, 1470, 1736, 1661, 1375, 1258, 1233, 1073, 1032 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 4; MS *m/z* 620.283 $[M]^+$ (2) (calcd for $C_{32}H_{44}O_{12}$, 620.283), 612 [M − H_2O]$^+$ (1), 560 (10), 501 (13), 83 (100).

(2*R,3*R**,4*S**,5*R**,9*S*[*R*]*,13*R**,15*R**)-2,3,5,15-Tetraacetoxy-9-hydroxy-8,14-dioxoeuphoperfolia-6,-11*E*-diene (21 and 22):** gum; ^1H and ^{13}C NMR data, see Table 5; MS *m/z* 548.226 $[M]^+$ (5) (calcd for $C_{28}H_{36}O_{11}$, 548.226), 488 [M − AcOH]$^+$ (3), 446 [488 − ketene]$^+$ (64), 386 [446 − AcOH]$^+$ (8), 326 [386 − AcOH]$^+$ (25), 262 (35), 202 (75), 123 (100).

12-*O*-Benzoyl-13-*O*-isobutyroyl-4-deoxyphorbol (23): gum; ^1H NMR ($CDCl_3$) δ 8.02 (2H, Bz AA′), 7.59 (Bz C), 7.46 (Bz BB′), 7.57 (1H, dq, *J* = 2.5, 1.5 Hz, H-1), 5.66 (1H, d, *J* = 10.0 Hz, H-12), 5.57 (1H, br d, *J* = 6.0 Hz, H-7), 4.06 (1H, d, *J* = 13.0 Hz, H-20a), 4.01 (1H, d, *J* = 13.0 Hz, H-20b), 3.29 (1H, ddq, *J* = 5.0, 2.5, 3.0 Hz, H-10), 2.87 (1H, br dd, *J* = 18.0, 10.0 Hz, H-5β), 2.61 (1H, m, 2-ibu), 2.52 (1H, ddd, *J* = 10.0, 10.0, 5.0 Hz, H-4), 2.45 (1H, br dd, *J* = 6.0, 5.5 Hz, H-8), 2.17 (1H, br dd, *J* = 18.0, 10.0 Hz, H-5α), 1.74 (1H, dq, *J* = 10.0, 6.5 Hz, H-11), 1.73 (3H, dd, *J* = 3.0, 1.5 Hz, H-19), 1.33 (3H, s, H-17), 1.21 (3H, s, H-16), 1.21 (3H, d, *J* = 7.0 Hz, ibu), 1.18 (3H, d, *J* = 7.0 Hz, ibu), 1.10 (1H, d, *J* = 5.5 Hz, H-14), 0.97 (3H, d, *J* = 6.5 Hz, H-18); MS *m/z* 522.263 $[M]^+$ (0.1) (calcd for $C_{31}H_{38}O_7$, 522.262), 504 [M − H_2O]$^+$ (0.2), 434 [M − *i*BuOH]$^+$ (2), 312 [434 − PhCOOH]$^+$ (6), 105 $[PhCO]^+$ (100).

12-*O*-Tigloyl-13-*O*-isobutyroyl-4-deoxyphorbol (24): gum; ^1H NMR ($CDCl_3$) δ 7.55 (1H, dq, *J* = 2.5, 1.5 Hz, H-1), 6.83 (1H, qq, *J* = 7.0, 1.5 Hz, tigl), 5.54 (1H, br d, *J* = 6.0 Hz, H-7), 5.45 (1H, d, *J* = 10.0 Hz, H-12), 4.05 (1H, d, *J* = 13.0 Hz, H-20a), 3.99 (1H, d, *J* = 13.0 Hz, H-20b), 3.25 (1H, ddq, *J* = 5.0, 2.5, 3.0 Hz, H-10), 2.84 (1H, br dd, *J* = 18.0, 10.0 Hz, H-5β), 2.58 (1H, m, 2-ibu), 2.48 (1H, ddd, *J* = 10.0, 10.0, 5.0 Hz, H-4), 2.39 (1H, br dd, *J* = 6.0, 5.5 Hz, H-8), 2.15 (1H, br dd, *J* = 18.0, 10.0 Hz, H-5α), 1.83 (3H, br d, *J* = 1.5 Hz, tigl), 1.80 (3H, dq, *J* = 7.0, 1.5 Hz, tigl), 1.73 (3H, dd, *J* = 3.0, 1.5 Hz, H-19), 1.60 (1H, dq, *J* = 10.0, 6.5 Hz, H-11), 1.23 (3H, s, H-17), 1.21 (3H, s, H-16), 1.19 (3H, d, *J* = 7.0 Hz, ibu), 1.16 (3H, d, *J* = 7.0 Hz, ibu), 1.04 (1H, d, *J* = 5.5 Hz, H-14), 0.91 (3H, d, *J* = 6.5 Hz, H-18); MS *m/z* 500.278 $[M]^+$(0.4) (calcd for $C_{29}H_{40}O_7$, 500.277), 482 [M − H_2O]$^+$ (0.4), 412 [M − *i*BuOH]$^+$ (2), 312 [412 − TiglOH]$^+$ (7), 83 $[C_4H_7CO]^+$ (100).

Acknowledgment. This work was supported by the EU (contract ERB FAIR CT 96 1781). V. M. is the recipient of an Erasmus fellowship. S. J. thanks Indena S.p.A. (Milano) for a fellowship. We are grateful to Dr. Raffaele Sestu and the Pro Loco Arzana for their hospitality and help during the collection of the plant material and to Dr. G. Höhne (T.U., Berlin) for the HREIMS measurements.

References and Notes

(1) Riddle, J. *ISIS* **1985**, *76*, 319−330.
(2) Hartwell, J. L. *Lloydia* **1969**, *32*, 153−205.
(3) Kupchan, S. M.; Uchida, I.; Branfman, A. R.; Dailey, R. G., Jr.; Yu Fei, B. *Science* **1976**, *191*, 571−572. Ingenol 3, 20-dibenzoate has a negligible affinity for protein kinase C, the target of tumor-promoting phorbol-type diterpenoids (Winkler, J. D.; Hong, B.-C.; Bahadar, A.; Kazanietz, M. G.; Blumberg, P. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 577−580).
(4) Evans, F. J.; Kinghorn, A. D. *J. Linn. Soc. (London, Bot.)* **1977**, *74*, 23−35. (b) Evans, F. J.; Taylor, S. E. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer: New York, 1983; Vol. 44, pp 1−99. (c) Singla, A. K.; Pathak, K. *Fitoterapia* **1990**, *61*, 483−516.
(5) Hecker, H. In *Pharmacognosy and Phytochemistry*; Wagner, H., Hörhammer, L., Eds.; Springer: Berlin, 1971; pp 147−165.
(6) For a recent example, see: Öksüz, S.; Gürek, F.; Yang, S.-W.; Lin, L.-Z.; Cordell, G. A.; Pezzuto, J. M.; Wagner, H.; Lotter, H. *Tetrahedron* **1997**, *53*, 3215−3222.
(7) In a particularly striking example, a single collection of *E. helioscopia* L. afforded 31 macrocyclic diterpenoids, the most abundant of which was isolated in only ca. 0.0006% yield. Yamamura, S.; Shizuri, Y.; Kosemura, S.; Ohtsuka, J.; Tayama, T.; Ohba, S.; Ito, M.; Saito, Y.; Terada, Y. *Phytochemistry* **1989**, *28*, 3421−3436.
(8) Analgesic and anticancer activities have been reported for macrocyclic diterpenoids from *Euphorbia* spp. (Uemura, B.; Katayama, C.; Luno, E.; Sasaki, K.; Hirata, Y. *Tetrahedron Lett.* **1975**, 1703−1706; Sahai, R.; Rastogi, R. P.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1981**, *20*, 1665−1667).
(9) Pignatti, S. *Flora d'Italia*; Edagricole: Bologna, 1982; Vol. 2, p 50.
(10) Turner, R. *Euphorbias*, Batsford: London, 1995; pp 77−81.
(11) Girin, M. A.; Paphassarang, S.; David-Eteve, Ch.; Chaboud, A.; Raynaud, J. *J. Chromatogr.* **1993**, *637*, 206−209.
(12) Shizuri, Y.; Kosemura, S.; Yamamura, S.; Ohba, S.; Ito, M.; Saito, Y. *Chem. Lett.* **1983**, 65−68.
(13) Jakupovic, J.; Morgenstern, T.; Marco, J. A.; Berendsohn, W. *Phytochemistry* (in press).
(14) Hohmann, J.; Vasas, A.; Günther, G.; Màthé, I.; Evanics, F.; Dombi, G.; Jerkovich, G. *J. Nat. Prod.* **1997**, *60*, 331−336.
(15) The bidimensional representation of the jatrophane skeleton offers the opportunity for a number of drawing modes and orientations. The absolute configuration of natural jatrophanes is such that, if the molecule is oriented with the five-membered ring to the left side of the observer, H-4 points away. An alternative representation, with the five-membered ring to the right side, also been employed.[7] In this case, H-4 points toward the observer.
(16) Jeske, F. *Naturstoffe aus Euphorbiaceen. Studien zum konformativen Verhalten von Jatrophanen*. Ph.D. Thesis, Technische Universität Berlin, 1996.
(17) Samek, Z.; Harmatha, J. *Collect. Czech. Chem. Commun.* **1978**, *43*, 2779−2799.
(18) Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Spectral Data for Structure Determination of Organic Compounds*; Springer: Berlin, 1989; p C190.
(19) Jakobi, P.; Haerle, E.; Schairer, H. U.; Hecker, E. *Liebigs Ann. Chem.* **1970**, *741*, 13−22.
(20) Fürstenberger, G.; Hecker, E. *Tetrahedron Lett.* **1977**, 925−928.
(21) Pax, F.; Hofmann, H. In *Die Natürliche Pflanzenfamilien*, 2nd ed.; Engelmann: Leipzig, 1931; p 208.
(22) For recent examples, see: (a) Appendino, G.; Jakupovic, J.; Alloatti, S.; Ballero, M. *Phytochemistry* **1997**, *45*, 1639−1643 and (b) Bianco, A.; Guiso, M.; Nicoletti, M.; Serafini, M.; Ballero, M. *Fitoterapia* **1996**, *67*, 364−366.
(23) Exhaustive studies on plants endemic to Sardinia were carried out by P. V. Arrigoni and published in a series (Le Piante Endemiche della Sardegna) that appeared in 202 monographs in *Boll. Soc. Sarda Sci. Nat.* during the years 1978−1991 (vol. 17−28).
(24) For a recent examples, see: Appendino, G.; Jakupovic, J.; Jakupovic, S. *Phytochemistry* **1997** *46*, 1039−1043.

NP970507W