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Jatrophane and tigliane diterpenes from the latex of *Euphorbia obtusifolia*

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Abstract

The latex of *Euphorbia obtusifolia* var. *obtusifolia* yielded twelve new diterpene polyesters. Seven of them displayed the jatrophane framework and five were 4-deoxyphorbol esters. A further isolated tigliane diterpene, a derivative of 4-*epi*-4-deoxyphorbol, was most likely an artifact of the isolation procedure. All structures were established with the aid of spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Euphorbia obtusifolia* var. *obtusifolia*; Euphorbiaceae; Diterpenes; Jatrophanes; Tiglianes; 4-deoxyphorbol esters

1. Introduction

Within the family Euphorbiaceae, the sixth largest among flowering plants, the genus *Euphorbia* L. alone accounts for almost a sixth of the whole group (Mabberley, 1987; Webster, 1994). In recent treatments of its true circumscription (Webster, 1994), well over 1000 species are ascribed to this genus. Many of them have been the object of chemical and pharmacological investigations because of the irritant and carcinogenic properties of their lattices (Evans & Taylor, 1983; Evans & Kinghorn, 1977; Singla & Pathak, 1990; Evans, 1986). These biological properties have been traced back in many cases to the presence of certain types of diterpenes, most particularly phorbol derivatives, which display the tigliane framework (Evans, 1986; Hickey, Worobec, West & Kinghorn, 1981). Further diterpenes belonging to other skeletal types, e.g. ingenane derivatives, are also characterized by similarly strong pharmacological effects (Evans, 1986).

2. Results and discussion

Within our recent interest in the phytochemistry of the genus *Euphorbia* (Marco, Sanz-Cervera, Yuste, Jakupovic & Lex, 1996; Marco, Sanz-Cervera, Yuste & Jakupovic, 1997a; Marco, Sanz-Cervera & Yuste, 1997b; Marco, Sanz-Cervera, Yuste, Jakupovic & Jeske, 1998a; Marco, Sanz-Cervera, Ropera, Checa & Fraga, 1998b; Jakupovic et al., 1998a; Jakupovic, Morgenstern, Marco & Berendsohn, 1998b), we have now investigated the chemical constituents of *E. obtusifolia* Poir. var. *obtusifolia* (syn. *E. broussonetii* Willd. ex Link in Buch), a member of the Macaronesian *E. obtusifolia* complex (Molero & Rovira, 1998; Oudejans, 1990). The latex of this species consisted mainly of widespread triterpenes (Singla & Pathak, 1990). We further isolated as minor components the jatrophane esters **1**–**7**, the 4-deoxyphorbol esters **8**–**12** and the 4-*epi*-4-deoxyphorbol ester **13**. The structures

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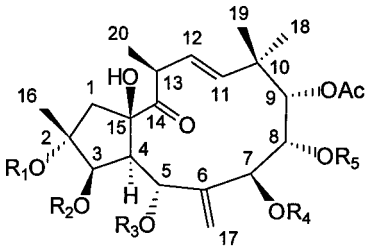
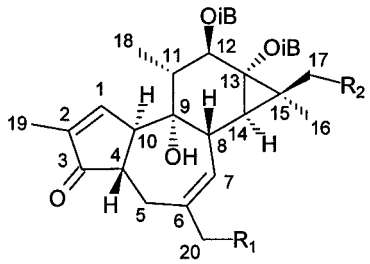


	R_1	R_2	R_3	R_4	R_5
1	MB	H	MB	Ac	Ac
2	iB	H	MB	Ac	Ac
3	Nic	H	MB	Ac	Ac
4	MB	H	MB	iB	Ac
5	Ac	H	MB	iB	Ac
6	MB	MB	H	Ac	Bz
7	MB	MB	H	iB	Ac



	R_1	R_2
8	H	H
9	OH	H
10	OH	OAc
11	H	OAc
12	OAc	H
13	(4-*epi*-**9**)	

MB = 2-methylbutyrate
iB = isobutyrate
Bz = benzoate
Nic = nicotinate

Table 1
^{1}H-NMR data of jatrophane derivatives **1**–**7**[a]

H	**1**	**2**[b]	**3**[c]	**4**[d]	**5**[e]	**6**[f]	**7**[g]
H-1α	2.79 *d* (16)	2.75 *d* (15.7)	2.93 *d* (15.7)	2.78 *d* (15.5)	2.70 *d* (15.5)	3.10 *d* (16)	3.04 *d* (16)
H-1β	2.07 *d* (16)	2.05 *d* (15.7)	2.27 *d* (15.7)	2.08 *d* (15.5)	2.03 *d* (15.5)	2.12 *d* (16)	2.10 *d* (16)
H-3	4.28 *dd* (9; 4.2)	4.32 *dd* (9; 4.5)	4.53 *dd* (9; 4.4)	4.24 *dd* (9.5; 4.4)	4.32 *dd* (9; 4.4)	5.74 *d* (4)	5.67 *d* (4.4)
H-4	3.05 *dd* (4.2; 2)	3.05 *dd* (4.5; 2)	3.19 *dd* (4.4; 2)	3.08 *dd* (4.4; 2)	3.08 *dd* (4.4; 2)	3.24 *dd* (4; 2)	3.26 *dd* (4.4; 2)
H-5	5.34 *br s*	5.34 *br s*	5.39 *br s*	5.34 *br s*	5.29 *br s*	4.25 *br s*	4.11 *br s*
H-7	5.41 *s*	5.42 *s*	5.36 *s*	5.43 *s*	5.52 *s*	5.45 *s*	5.44 *s*
H-8	5.09 *s*[h]	5.09 *s*	5.10 *s*	5.11 *s*	5.09 *s*	5.16 *s*	4.88 *s*
H-9	4.92 *s*	4.92 *s*	4.90 *s*	4.94 *s*	4.95 *s*	4.98 *s*	4.86 *s*
H-11	5.83 *d* (15.7)	5.86 *d* (15.6)	5.72 *d* (15.7)	5.88 *d* (16)	5.97 *d* (16)	5.87 *d* (16)	5.86 *d* (16)
H-12	5.50 *dd* (15.7; 9.5)	5.50 *dd* (15.6; 9.5)	5.46 *dd* (15.7; 9.5)	5.50 *dd* (16; 9.5)	5.48 *dd* (16; 9.5)	5.43 *dd* (16; 9.5)	5.35 *dd* (16; 9.7)
H-13	3.68 *dq* (9.5; 7)	3.70 *dq* (9.5; 6.5)	3.62 *dq* (9.5; 7)	3.70 *dq* (9.5; 6.5)	3.78 *dq* (9.5; 6.5)	3.72 *dq* (9.5; 6.5)	3.73 *dq* (9.7; 6.5)
H-16	1.70 *s*	1.69 *s*	1.85 *s*	1.70 *s*	1.70 *s*	1.55 *s*	1.53 *s*
H-17	5.26 *br s*	5.26 *br s*	5.29 *br s*	5.25 *br s*	5.24 *br s*	5.18 *br s*	5.23 *t* (1.5)
	5.09 *br s*[h]	5.09 *br s*	5.12 *br s*	5.11 *br s*	5.13 *br s*	5.05 *br s*	5.12 *br d* (1.5)
H-18	0.92 *s*	0.92 *br s*	0.85 *s*	0.92 *br s*	0.93 *br s*	0.94 *s*	0.90 *br s*
H-19	1.28 *s*	1.28 *br s*	1.26 *s*	1.30 *br s*	1.31 *br s*	1.21 *s*	1.16 *br s*
H-20	1.22 *d* (7)	1.24 *d* (6.5)	1.19 *d* (7)	1.22 *d* (6.5)	1.26 *d* (6.5)	1.27 *d* (6.5)	1.24 *d* (6.5)
3/5-OH	3.30 *d* (9)	3.30 *d* (9)	3.35 *d* (9)	3.30 *d* (9.5)	3.35 *d* (9)	4.50 *s*	4.40 *s*
15-OH	4.30 *s*	4.30 *s*	4.35 *s*	4.30 *s*	4.30 *s*	5.00 *s*	4.95 *s*
OAc	2.11 *s*, 2.05 *s*	2.11 *s*, 2.05 *s*	2.13 *s*, 2.05 *s*	2.02 *s*, 2.00 *s*	2.16 *s*, 2.03 *s*	2.16 *s*, 2.09 *s*	2.02 *s* (6H)
	2.00 *s*	2.00 *s*	2.00 *s*		2.00 *s*		
OMB	2.50 *tq*, 2.34 *tq* (7; 7)	2.34 *tq* (7; 7)	2.36 *tq* (7; 7)	2.51 *tq*, 2.34 *tq* (7; 7)	2.34 *tq* (7; 7)	2.48 *tq*, 2.34 *tq* (7; 7)	2.50 *tq*, 2.35 *tq* (7; 7)
	1.80-1.40 *m*	1.65 *m*	1.68 *m*	1.80-1.40 *m*	1.65 *m*	1.85 *m*, 1.70 *m*	1.90-1.70 *m*
	1.18 *d* (7)	1.43 *m*	1.45 *m*	1.20 *d* (7)	1.43 *m*	1.55 *m*, 1.40 *m*	1.60-1.40 *m*
	1.10 *d* (7)	1.10 *d* (7)	1.13 *d* (7)	1.10 *d* (7)	1.10 *d* (7)	1.25 *d* (7), 1.13 *d* (7)	1.25 *d* (7), 1.15 *d* (7)
	0.96 *t* (7.5)	0.85 *t* (7.5)	0.88 *t* (7.5)	0.95 *t* (7.5)	0.85 *t* (7.5)	0.97 *t* (7.5)	0.96 *t* (7.5), 0.89 *t* (7.5)
	0.86 *t* (7.5)			0.86 *t* (7.5)		0.86 *t* (7.5)	

[a] δ in ppm and *J* (parentheses) in Hz (400 MHz, $CDCl_3$, 22°C).
[b] iB: 2.65 *qq* (7; 7), 1.23 *d*, 1.20 *d* (7).
[c] Nic: 9.40 *br s*, 8.78 *br s*, 8.46 *br d* (8), 7.40 *br t* (6.5).
[d] iB: 2.60 *qq* (7; 7), 1.20 *d*, 1.16 *d* (7).
[e] iB: 2.60 *qq* (7; 7), 1.19 *d*, 1.15 *d* (7).
[f] Bz: 8.00 *dd* (8; 1.5), 7.52 *tt* (8; 1.5), 7.40 *td* (8; 1.5).
[g] iB: 2.60 *qq* (7; 7), 1.23 *d*, 1.16 *d* (7).
[h] Overlapped signals.

Table 2
^{13}C-NMR data of jatrophane derivatives **1**–**7**[a]

C	**1**	**2**[b]	**3**[c]	**4**[d]	**5**[e]	**6**[f]	**7**[g]
C-1	50.5	50.7	50.5	50.5	51.6	49.2	49.3
C-2	89.6	89.6	91.8	89.6	89.8	89.0[h]	88.9
C-3	78.8	78.5	79.0	79.1	77.8	77.8	77.9
C-4	47.5	47.5	47.5	47.4	47.3	48.3	48.2
C-5	68.3	68.4[h]	68.3	68.3	68.3	68.7	68.8
C-6	144.5	144.5[i]	144.5[i]	145.1	145.5[i]	144.1	145.2
C-7	68.5	68.3[h]	68.8	67.8	67.5	68.7	67.7
C-8	69.9	69.9	69.9	70.2	70.5	70.5	70.2
C-9	80.4	80.4	80.3	80.4	80.3	80.4	80.2
C-10	40.8	40.9	40.8	40.9	40.7	41.0	41.0
C-11	137.2	137.3	137.4	137.4	137.8	137.1	137.3
C-12	129.7	129.6	129.5	129.6	129.2	129.7	129.3
C-13	44.0	44.0	44.1	44.0	44.0	44.1	44.0
C-14	211.5	211.5	211.2	211.5	211.8	211.9	212.1
C-15	88.4	88.3	88.5	88.6	88.3	88.9[h]	89.2
C-16	20.1	20.1	20.3	20.2	19.7	19.3	19.5
C-17	111.5	111.5[i]	111.5[i]	111.1	111.5[i]	112.7	112.3
C-18	26.1	26.1	26.0	26.3	26.2	25.8	25.9
C-19	23.0	23.0	22.9	23.0	22.5	22.5	22.6
C-20	19.8	19.7	19.6	19.6	19.5	19.6	19.5
OAc	169.7, 169.4, 168.8	169.7, 169.4, 168.9	169.6, 169.5, 169.4	169.7, 169.5	170.5, 169.8, 169.6	169.3, 168.6	170.1, 169.5
	21.0, 20.6, 20.5	21.0, 20.6, 20.5	21.0, 20.6, 20.5	20.7, 20.5	20.7, 20.6, 20.5	21.0, 20.5	20.6, 20.5
OMB	176.2, 174.2	174.2	174.2	176.2, 174.3	174.3	175.7, 174.5	175.8, 174.5
	41.7, 41.3	41.3	41.3	41.8, 41.3	41.3	42.3, 41.2	42.3, 41.3
	26.7, 26.4	26.3	26.4	26.5, 26.4	26.3	26.6, 26.0	26.4, 26.0
	16.3, 16.1	16.3	16.3	16.4, 16.3	16.3	16.4, 16.3	16.5, 16.4
	11.8, 11.5	11.5	11.5	11.8, 11.5	11.5	12.0, 11.6	12.0, 11.7

[a] δ in ppm (100 MHz, $CDCl_3$, 22°C). Signals assigned by means of 2D-NMR experiments.
[b] iB: 176.5, 34.8, 19.0, 18.6.
[c] Nic: 164.7, 153.3, 151.3, 144.5, 137.4, 123.1.
[d] iB: 174.9, 33.7, 19.5, 18.3.
[e] iB: 175.4, 33.7, 19.5, 18.3.
[f] Bz: 165.3, 133.2, 129.9, 129.4, 128.3.
[g] iB: 174.9, 33.7, 19.6, 18.3.
[h] Signals with this superscript are interchangeable within the same spectrum.
[i] Not emerged from the background (localized through the HMQC spectrum).

of these compounds were established through extensive 1D (spin decoupling, NOE) and 2D (HMQC, HMBC) NMR measurements. The configurations at the stereogenic centres were deduced from coupling constants values, NOE measurements and comparison with literature data. Compounds **11** and **12** could not be separated with our chromatographic systems. Their NMR signals were sorted out and assigned by comparison with the remaining compounds and with literature data. Since 4-deoxyphorbol derivatives are known to be prone to epimerization at C-4 (Evans, 1986), compound **13** is most likely an artifact of the isolation procedure.

Compounds **1**–**7** belong to the jatrophane group of diterpenes and share all a common arrangement of functional groups. These structural features were easily identified through the close similarity of their NMR spectra (Tables 1 and 2) with those of jatrophane polyesters recently reported in *E. terracina, E. segetalis, E. paralias, E. peplus* and *E. semiperfoliata* (Marco et al., 1998a; Jakupovic et al., 1998a; 1998b; Jakupovic, Morgenstern, Bittner & Silva, 1998c; Appendino et al., 1998). Some of these and all the compounds now under study are polyacylated derivatives of the same parent jatrophane having a ketone carbonyl group at C-14, seven hydroxyl groups at C-2, C-3, C-5, C-7, C-8, C-9 and C-15, a *trans* double bond at C-11/C-12, and an *exo*-methylene group at C-6. This was confirmed again in the present case by thorough NMR measurements, with particular emphasis on HMBC experiments. These served to establish the carbon framework in an unequivocal way and to locate the different acyl residues, which belonged to five structural types: acetate, isobutyrate, 2-methylbutyrate, benzoate and nicotinate. NOE measurements were used to confirm the stereochemical arrangement of the stereogenic centres, which turned out to be the same as in the jatrophanes from *E. terracina* (Marco et

Table 3
^{1}H-NMR data of 4-deoxyphorbol derivates **8**–**13**[a]

H	8	9	10[b]	11[b]	12[b]	13
H-1	7.56 *br s*	7.55 *br s*	7.50 *br s*	7.51 *br s*	7.55 *br s*	7.05 *br s*
H-4	2.43 *ddd* (10.5; 9; 4.5)	2.46 *ddd* (10; 9.5; 4.5)	2.47 *ddd* (10; 9.5; 4.5)	2.44 *m*	2.44 *m*	2.78 *ddd* (5.2; 2.5; 2.5)
H-5α	2.00 *dd* (18.2; 10.5)	2.14 *dd* (18; 10)	2.10 *dd* (18; 10)	2.00 *dd* (18; 10)	2.13 *dd* (18; 10)	2.47 *dd* (15.5; 5.2)
H-5β	2.82 *br dd* (18.2; 9)	2.82 *br dd* (18; 9.5)	2.73 *br dd* (18; 9.5)	2.74 *br dd* (18; 9)	2.84 *br dd* (18; 9)	3.45 *dddd* (15.5; 2.5; 2.5; 2.5)
H-7	5.21 *br dq* (5.5; 2)	5.54 *br dq* (5.5; 2)	5.52 *br dq* (5.5; 2)	5.20 *br dq* (5.5; 2)	5.55 *m*[c]	5.10 *br s*
H-8	2.32 *br t* (5.5)	2.36 *br t* (5.5)	2.33 *br t* (5.5)	2.25 *br t* (5.5)	2.37 *br t* (6)	1.95 *m*
H-10	3.28 *dddq* (4.5; 2.5; 2.5; 1.5)	3.24 *dddq* (4.5; 2.5; 2.5; 1.5)	3.20 *dddq* (4.5; 2.5; 2.5; 1.5)	3.24 *dddq* (4.5; 2.5; 2.5; 1.5)	3.24 *dddq* (4.5; 2.5; 2.5; 1.5)	3.50 *dddq* (2.5; 2.5; 2.5; 1.5)
H-11	1.55 *dq* (9.5; 6.5)	1.56 *dq* (10; 6.5)	1.67 *dq* (10; 6.5)	1.60 *dq* (9.5; 6.5)	1.60 *dq* (9.5; 6.5)	1.68 *dq* (10.5; 6.5)
H-12	5.37 *d* (9.5)	5.38 *d* (10)	5.40 *d* (10)	5.38 *d* (9.5)	5.38 *d* (9.5)	5.41 *d* (10.5)
H-14	0.96 *d* (5.5)	1.03 *d* (5.5)	1.20 *d* (5.5)	1.15 *m*[c]	1.00 *d* (6)	0.75 *d* (5)
H-16	1.20 *s*	1.20 *s*	1.29 *s*	1.29 *s*	1.20 *s*	1.16 *s*
H-17	1.20 *s*	1.20 *s*	4.50 *d* (12.5) 4.08 *d* (12.5)	4.48 *d* (12.5) 4.07 *d* (12.5)	1.20 *s*	1.20 *s*
H-18	0.89 *d* (6.5)	0.90 *d* (6.5)	0.93 *d* (6.5)	0.92 *d* (6.5)	0.90 *d* (6.5)	1.06 *d* (6.5)
H-19	1.72 *br s*	1.72 *dd* (2.5; 1.5)	1.72 *dd* (2.5; 1.5)	1.72 *t* (1.5)	1.72 *t* (1.5)	1.78 *t* (1.5)
H-20	1.72 *br s*	4.01 *br d* (15) 3.99 *br d* (15)	4.00 *br s* (2H)	1.73 *dd* (2; 1.5)	4.46 *br d* (12.5) 4.42 *br d* (12.5)	4.00 *br d* (12) 3.88 *br d* (12)
9-OH	5.70 *br s*	5.80 *br s*	5.60 *br s*	5.50 *br s*	5.80 *br s*	5.30 *br s*
OiB	2.55 *qq* (7; 7) 2.54 *qq* (7; 7) 1.17 *d* (7) 1.16 *d* (7) 1.15 *d* (7) 1.14 *d* (7)	2.56 *qq* (7; 7) 2.55 *qq* (7; 7) 1.17 *d* (7) 1.16 *d* (7) 1.15 *d* (7) 1.14 *d* (7)	2.58 *qq* (7; 7) 2.54 *qq* (7; 7) 1.18 *d* (7) 1.17 *d* (7) 1.16 *d* (7) 1.15 *d* (7)	2.55 *qq* (7; 7) 2.53 *qq* (7; 7) 1.18 *d* (7) 1.16 *d* (7) 1.14 *d* (7) 1.12 *d* (7)	2.55 *qq* (7; 7) 2.53 *qq* (7; 7) 1.18 *d* (7) 1.16 *d* (7) 1.14 *d* (7) 1.12 *d* (7)	2.60 *qq* (7; 7) 2.52 *qq* (7; 7) 1.21 *d* (7) 1.19 *d* (7) 1.15 *d* (7) 1.12 *d* (7)

[a] δ in ppm and J (parentheses) in Hz (400 MHz, $CDCl_3$, 22°C).
[b] OAc: 2.05 *s*.
[c] Overlapped signal.

al., 1998a) and *E. segetalis* (Jakupovic et al., 1998a). The small value of $J_{4,5}$ further indicates that jatrophanes **1**–**7** display predominantly a conformation with the *exo*-methylene group pointing outwards and H-5 pointing inwards in relation to the twelve-membered ring (Marco et al., 1998a; Jakupovic et al., 1998a; 1998b; Jakupovic, Morgenstern, Bittner & Silva, 1998c; Appendino et al., 1998).

The NMR spectra of compounds **8**–**12** (Tables 3 and 4) showed typical signals of phorbol esters (Evans, 1986). Most characteristic were the ^{1}H doublets at ca 5.40 ppm ($J \sim 9$–10 Hz) and at ca 1 ppm ($J \sim 5$ Hz), attributed to H-12 and H-14, respectively. In the ^{13}C NMR spectra, signals from the *gem*-dimethylcyclopropane moiety were visible at about 65 and 25–30 ppm (both quaternary). All compounds displayed two isobutyrate groups, which were located at C-12 and C-13 because, respectively, of the distinct HMBC correlation (12-OiB) and characteristic carbonyl chemical shift (13-OiB). Compounds **8**, **9** and **12** had an unfunctionalized *gem*-dimethylcyclopropane fragment, which gave rise to a six-proton singlet at 1.20 ppm. In addition, **8** and **11** had two olefinic methyl groups (H-19 and H-20), which were visible as two almost coincident three-proton signals. In **9**, **10** and **12**, C-20 was functionalized with an OH or OAc group, a feature which gave rise to characteristic NMR signals in the middle range (3.50–4.50 ppm) of the ^{1}H NMR spectrum. In the same way, the observation of only one methyl singlet at high-field (1.29 ppm) and of new signals in the middle range of the spectra indicated that **10** and **11** had an oxygen atom at one of the *gem*-dimethylcyclopropane methyl groups. The observation of NOE between H-11 and the protons vicinal to the oxygen atoms, as well as between H-14 and the methyl group at δ 1.29, indicated that this oxygen function resided at C-17. All other NOE were completely consistent with the phorbol-type configuration: those observed in H-10 with H-7 and with 9-OH confirmed the α arrangement of H-10 while the NOE between H-4 and H-8 established the β orientation of H-4.

Compound **13**, isolated in a very small amount, displayed some differences in its NMR spectral data with the other phorbol derivatives. While the same functional fragments as in **9** seemed to be present, characteristic differences in the chemical shifts of some ^{1}H

Table 4
^{13}C-NMR data of 4-deoxyphorbol derivatives **8–13**[a]

C	8	9	10	11	12	13
C-1	160.2	159.8	160.0	159.4	159.8	156.2
C-2	136.3	136.4	136.6	136.5	136.5	143.3
C-3	210.2	209.7	209.7	210.1	209.6	213.3
C-4	44.5	44.2	44.0	44.3	44.1	49.6
C-5	34.0	29.6	29.0	33.6	30.0	25.1
C-6	139.0	142.0	142.4	139.3	137.2	137.0
C-7	125.8	126.5	125.3	125.0	130.3	126.5
C-8	42.2[b]	42.1[b]	42.4	42.6[b]	42.2[b]	40.7
C-9	77.9	77.8	–[c]	77.5	77.8	78.1
C-10	54.3	54.2	53.8	53.9	54.0	47.4
C-11	42.3[b]	42.4[b]	42.4	42.5[b]	42.3[b]	43.2
C-12	76.7	76.7	76.1	76.2	76.4	75.3
C-13	65.0	65.0	65.2	65.3	64.8	64.8
C-14	35.9	35.8	36.4	36.5	35.5	37.1
C-15	25.8	25.9	30.0	29.8	25.8	25.3
C-16	23.9	23.8	–[d]	19.5	23.8	24.2
C-17	16.9	16.9	63.3	63.5	16.8	16.5
C-18	15.1	15.1	15.2	15.2	15.0	11.9
C-19	10.2	10.2	10.3	10.3	10.3	10.5
C-20	25.4	67.5	67.1	25.4	68.9	69.3
OAc	–	–	171.0	171.0	169.3	–
			20.8	20.8	21.0	
OiB	179.2, 176.5	179.3, 176.5	179.1, 176.4	179.0, 176.4	179.3, 176.3	179.1, 176.5
	34.2, 34.1	34.2 (×2)	34.2, 34.1	34.2, 34.1	34.2, 34.1	34.3, 34.2
	19.0, 18.9	19.1, 18.9	19.4, 19.1	19.0, 18.9	19.0, 18.9	19.2, 18.8
	18.6, 18.5	18.6, 18.5	18.9, 18.6	18.8, 18.5	18.8, 18.5	18.6, 18.5

[a] δ in ppm (100 MHz, $CDCl_3$, 22°C). Signals assigned by means of 2D-NMR experiments.
[b] Signals with this superscript are interchangeable within the same spectrum.
[c] Obscured by the signals of $CDCl_3$.
[d] Overlaps with one of the isobutyrate methyl signals.

signals, most particularly H-1, H-5, H-7 and H-10, suggested it to be a stereoisomer of the latter compound (Evans, 1986). Changes in the values of the coupling constants $J_{4,5\alpha}$, $J_{4,5\beta}$ and $J_{4,10}$ pointed at a change in configuration at C-4 or at C-10. That the configuration at C-4 was opposite to that present in 4-deoxyphorbols was finally deduced from the observation of NOE between H-4 and H-10, as well as from the absence of NOE between H-4 and H-8. Compound **13** was therefore an ester of 4-*epi*-4-deoxyphorbol. In all likelihood, however, it is not a natural product, but rather an artifact produced by epimerization of **9** during the isolation and chromatographic separation. There is ample precedent of such epimerizations taking place at C-4 of 4-deoxyphorbol derivatives (Evans, 1986)

3. Experimental

3.1. General

NMR (22°): in $CDCl_3$ at 400 (^{1}H) and 100 MHz (^{13}C). The solvent signals were taken as the reference. EIMS (70 eV) and FABMS (product dispersed in a *m*-nitrobenzyl alcohol matrix) were measured in a VG AutoSpec mass spectrometer. Optical rotations in $CHCl_3$ at 22°. Normal pressure CC on silica gel Südchemie AG (particle size 60–200 μ). Reverse-phase silica gel: silanized silica gel Merck (Art. 07719). HPLC: LiChrosorb RP-8 (250 × 8 mm), elution with MeOH–H_2O mixtures.

3.2. Plant material

The latex of *E. obtusifolia* var. *obtusifolia* (ca 330 g) was collected in May 1996 from specimens growing in the Valle de Tabares, Teneriffa. The plant material was authenticated by Dr Arnoldo Santos, from the Botanical Acclimatization Garten at La Orotava, Teneriffa, Canary Islands (voucher number: ORT-33458).

3.3. Extraction and chromatography

The latex was suspended in boiling MeOH (1.5 l) and then re-cooled to room temperature. This gave rise to a voluminous, whitish precipitate consisting

mainly of waxes and triterpenes, which were eliminated by filtration. Evaporation of the solvent in vacuo gave a whitish, oily material (ca 22.8 g). After dissolving this oil in the minimum amount of methanol, reverse-phase silica gel was added (3 g of silica gel/g of extract) (Marco et al., 1996; 1997a; 1997b; 1998a). The solvent was then totally eliminated in vacuo. The powdery material obtained was placed on the top of a chromatographic column filled with the same type of silica gel and eluted under a slight argon pressure (1.5–2 atm) first with water (3 l), then with methanol–water 70:30 (2 l) and finally with methanol (2 l). The water and methanol fractions only contained polar, ill-defined compounds and common triterpenes, respectively, and were discarded. The middle fraction was concentrated in vacuo to eliminate most MeOH and then extracted with EtOAc. The organic layer was dried on sodium sulphate and concd. in vacuo. This yielded an oil (3.32 g) which was then subjected to further chromatographic separations as described below.

The middle fraction was subjected to CC on silica gel (elution with hexane–EtOAc 5:1 → EtOAc, then EtOAc–MeOH 1:1). The intermediate fractions were further purified, where necessary, by prep. TLC and/or HPLC. This allowed the isolation of the compounds mentioned in the text. These were eluted from the silica gel column in the following order of increasing polarity: **8** (5 mg), **6** (6 mg), **7** (10 mg), **4** (4 mg), **11**/**12** (9 mg), **1** (10 mg), **5** (5 mg), **2** (5 mg), **13** (2 mg), **9** (9 mg), **3** (4 mg) and **10** (7 mg).

*3.4. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-7,8,9-triacetate-2,5-bis(2-methylbutyrate) (**1**)*

Oil, $[\alpha]_D + 23^\circ$ ($CHCl_3$; *c* 0.78); IR ν_{max}^{film} cm^{-1}: 3450 (*br*, OH), 1747 (*br*, ester and ketone C=O), 1458, 1374, 1251, 1225, 1138, 1069, 1043, 963; EIMS (probe) *m/z* (rel. int.): 694.3565 $[M]^+$ (3), 552 (57), 462 (75), 402 (64), 342 (57), 300 (59), 282 (100), 221 (80). Calc. for $C_{36}H_{54}O_{13}$, $M_r = 694.3564$; NMR, Tables 1 and 2.

*3.5. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-7,8,9-triacetate-2-isobutyrate-5-(2-methylbutyrate) (**2**)*

Oil, $[\alpha]_D + 29^\circ$ ($CHCl_3$; *c* 0.68); IR ν_{max}^{film} cm^{-1}: 3430 (*br*, OH), 1743 (*br*, ester and ketone C=O), 1453, 1374, 1225, 1139, 1069, 1043; EIMS (probe) *m/z* (rel. int.): 680.3402 $[M]^+$ (3), 552 (37), 462 (54), 402 (43), 342 (47), 300 (55), 282 (100), 221 (76). Calc. for $C_{35}H_{52}O_{13}$, $M_r = 680.3408$; NMR, Tables 1 and 2.

*3.6. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-7,8,9-triacetate-2-nicotinate-5-(2-methylbutyrate) (**3**)*

Oil, $[\alpha]_D - 6^\circ$ ($CHCl_3$; *c* 0.68); IR ν_{max}^{film} cm^{-1}: 3450 (*br*, OH), 1741, 1718 *sh* (*br*, ester and ketone C=O), 1375, 1289, 1224, 1114, 1069, 1042, 734; EIMS (probe) *m/z* (rel. int.): 715.3198 $[M]^+$ (100), 552 (35), 526 (71), 462 (36), 402 (29), 342 (32), 300 (37), 282 (88), 221 (43). Calc. for $C_{37}H_{49}NO_{13}$, $M_r = 715.3204$; NMR, Tables 1 and 2.

*3.7. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-8,9-diacetate-7-isobutyrate-2,5-bis(2-methylbutyrate) (**4**)*

Oil, $[\alpha]_D + 26^\circ$ ($CHCl_3$; *c* 0.86); IR ν_{max}^{film} cm^{-1}: 3450 (*br*, OH), 1749, 1734 (*br*, ester and ketone C=O), 1458, 1374, 1224, 1184, 1139, 1088, 1068, 1038; EIMS (probe) *m/z* (rel. int.): 722.3876 $[M]^+$ (3), 580 (28), 490 (33), 402 (39), 342 (42), 300 (53), 282 (100), 249 (62). Calc. for $C_{38}H_{58}O_{13}$, $M_r = 722.3877$; NMR, Tables 1 and 2.

*3.8. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-2,8,9-triacetate-7-isobutyrate-5-(2-methylbutyrate) (**5**)*

Oil, $[\alpha]_D + 32^\circ$ ($CHCl_3$; *c* 0.68); IR ν_{max}^{film} cm^{-1}: 3410 (*br*, OH), 1747, 1734 (*br*, ester and ketone C=O), 1374, 1242, 1225, 1143, 1086, 1039; EIMS (probe) *m/z* (rel. int.): 680.3423 $[M]^+$ (8), 580 (35), 490 (28), 402 (34), 342 (50), 300 (55), 282 (100), 249 (50). Calc. for $C_{35}H_{52}O_{13}$, $M_r = 680.3408$; NMR, Tables 1 and 2.

*3.9. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-7,9-diacetate-8-benzoate-2,3-bis(2-methylbutyrate) (**6**)*

Oil, $[\alpha]_D + 18^\circ$ ($CHCl_3$; *c* 0.88); IR ν_{max}^{film} cm^{-1}: 3420 (*br*, OH), 1728 (*br*, ester and ketone C=O), 1373, 1236; EIMS: no parent peak visible. FABMS *m/z* 779.3637 $[M^+ + Na]$. Calc. for $C_{41}H_{56}O_{13}Na$, $M_r = 779.3618$; NMR, Tables 1 and 2.

*3.10. (2R,3R,4R,5R,7S,8S,9S,11E,13S,15R)-2,3,5,7,8,9,15-Heptahydroxyjatropha-6(17),11-diene-14-one-8,9-diacetate-7-isobutyrate-2,3-bis(2-methylbutyrate) (**7**)*

Oil, $[\alpha]_D + 8^\circ$ ($CHCl_3$; *c* 2.2); IR ν_{max}^{film} cm^{-1}: 3450 (*br*, OH), 1738 (*br*, ester and ketone C=O), 1373,

1224; EIMS (probe) *m/z* (rel. int.): 722.3897 $[M]^+$ (2), 620 (4), 574 (5), 486 (12), 430 (20), 402 (20), 342 (22), 300 (30), 282 (62), 105 (40), 85 (54), 57 (100). Calc. for $C_{38}H_{58}O_{13}$, $M_r = 722.3877$; NMR, Tables 1 and 2.

3.11. 4,20-Dideoxyphorbol 12,13-bis(isobutyrate) (**8**)

Oil, $[\alpha]_D + 54^\circ$ ($CHCl_3$; *c* 0.74); IR ν_{max}^{film} cm^{-1}: 3400 (*br*, OH), 1735, 1717, 1708 (*br*, ester and ketone C=O), 1469, 1387, 1375, 1191, 1171, 908; EIMS (probe) *m/z* (rel. int.): 472.2823 $[M]^+$ (1), 401 (9), 384 (21), 296 (12), 71 (100). Calc. for $C_{28}H_{40}O_6$, $M_r = 472.2825$; NMR, Tables 3 and 4.

3.12. 4-Deoxyphorbol 12,13-bis(isobutyrate) (**9**)

Oil, $[\alpha]_D + 47^\circ$ ($CHCl_3$; *c* 0.64); IR ν_{max}^{film} cm^{-1}: 3400 (*br*, OH), 1734, 1716, 1702 (*br*, ester and ketone C=O), 1466, 1375, 1191, 1160, 979; EIMS (probe) *m/z* (rel. int.): 488.2777 $[M]^+$ (1), 470 (2), 445 (4), 401 (29), 312 (22), 71 (100). Calc. for $C_{28}H_{40}O_7$, $M_r = 488.2774$; NMR, Tables 3 and 4.

3.13. 17-Acetoxy-4-deoxyphorbol 12,13-bis(isobutyrate) (**10**)

Oil, $[\alpha]_D + 70^\circ$ ($CHCl_3$; *c* 1.2); IR ν_{max}^{film} cm^{-1}: 3410 (*br*, OH), 1737, 1730, 1713 (*br*, ester and ketone C=O), 1469, 1388, 1375, 1226, 1160, 1029, 989; EIMS (probe) *m/z* (rel. int.): 546.2826 $[M]^+$ (3), 528 (5), 458 (8), 398 (17), 310 (48), 190 (35), 71 (100). Calc. for $C_{30}H_{42}O_9$, $M_r = 546.2829$; NMR, Tables 3 and 4.

3.14. 17-Acetoxy-4,20-dideoxyphorbol 12,13-bis(isobutyrate) (**11**) and 4-deoxyphorbol 12,13-bis(isobutyrate) 20-acetate (**12**)

(Mixture which could not be separated): IR ν_{max}^{film} cm^{-1}: 3400 (*br*, OH), 1737, 1734, 1716 (*br*, ester and ketone C=O); EIMS (probe) *m/z* (rel. int.): 470.2676 $[M-HOAc]^+$ (4), 442 (9), 311 (15), 294 (38), 71 (100). Calc. for $C_{30}H_{42}O_8$–HOAc, $M_r = 470.2668$; NMR, Tables 3 and 4.

3.15. 4-Epi-4-deoxyphorbol 12,13-bis(isobutyrate) (**13**)

Oil, $[\alpha]_D + 3^\circ$ ($CHCl_3$; *c* 0.7); IR ν_{max}^{film} cm^{-1}: 3410 (*br*, OH), 1748, 1733, 1717 (*br*, ester and ketone C=O), 1250, 1194, 1158; EIMS (probe) *m/z* (rel. int.): 488.2760 $[M]^+$ (1), 470 (14), 445 (6), 417 (15), 400 (31), 312 (87), 294 (100), 71 (83). Calc. for $C_{28}H_{40}O_7$, $M_r = 488.2774$; NMR, Tables 3 and 4.

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