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DITERPENES FROM THE LATEX OF *EUPHORBIA BROTERI*

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Key Word Index—*Euphorbia broteri*; Euphorbiaceae; latex; proinflammatory fractions; ingenol and phorbol mono-, di- and triesters; tetracyclic diterpenes.

Abstract—Six polycyclic diterpenes have been isolated from the latex of *Euphorbia broteri* Daveau. Two had a tigliane skeleton: 12-*O*-(2*Z*,4*E*-octadienoyl)-4-deoxyphorbol-13,20-diacetate and 12-*O*-(2*Z*,4*E*-octadienoyl)-phorbol-13,20-diacetate and four an ingenane skeleton: 20-acetyl-ingenol-3-decadienoate, 3-*O*-tetradecanoyl-ingenol, 20-*O*-tetradecanoyl-ingenol and 5-*O*-tetradecanoyl-ingenol. The second and last two compounds are described as natural compounds for the first time. Their structures were established by spectroscopic methods, by chemical correlations and by H/H and C/H correlations in their ^{1}H NMR and ^{13}C NMR spectra.

INTRODUCTION

In recent years considerable attention has been devoted to the isolation from the latex of plants from the genus *Euphorbia* of compounds with pro-inflammatory and co-carcinogenic activities [1]. We were thus prompted to initiate a study of *Euphorbia broteri* Daveau which is a native of the Iberian Peninsula. Preliminary studies of the chemical compounds of this plant [2] have revealed the presence of triterpene compounds.

RESULTS AND DISCUSSION

The latex of *E. broteri* (Bèjar, Salamanca, Spain) was collected drop by drop in methanol and the irritant fraction isolated as described in the Experimental. CC of this fraction gave compounds **1**–**6**.

The ^{1}H NMR spectrum of **1** showed signals of a derivative of ingenol with two of its hydroxyl groups esterified [δ5.58 (1H, *s*, H-3) and δ4.78 and 4.48 (2H, AB, J = 12.5 Hz]. The displacement of the AB system was characteristic of H-20 when the C-5 hydroxyl group is free [3]. The hydroxyl groups were esterified with acetic acid (2.06, *s*, 3H) and a deca-2,4-dienoic acid (see Tables 1 and 2).

Alkaline hydrolysis of **1** followed by esterification of the acid fraction gave (2*Z*,4*E*)-methyl-decadienoate (**1a**) which on catalytic hydrogenation formed methyl caproate (identified by GC). The positions of the acyl groups were fixed according to the literature [3, 4], such that it was possible to assign to compound **1** a structure of 20-acetyl-ingenol-3-*O*-(2*Z*,4*E*)-decadienoate [5]. This structure, like those of the acid moieties, was confirmed by mass spectroscopy, which revealed the presence of a molecular ion of *m/z* 541 $[M+1]^+$ concordant with a formula of $C_{32}H_{44}O_7$ and fragments of *m/z* 480 $[M-AcOH]^+$ and 372 $[M-RCOOH]^+$. The signals in the ^{13}C NMR spectrum were assigned by comparison with those assigned to **3** and **5** by means of two dimensional correlation ^{13}C–^{1}H experiments. Compound **2** (Tables 3 and 4) was a derivative of phorbol (7.55, *q*, J = 1.46, H-1) with two acetoxyl groups (2.05 and 2.11, *s*, 3H, each) and an acid moiety with a different value of n to the one in **1**. The ^{13}C NMR spectrum showed signals of 32 carbon atoms, four of them methylenes. This established that in the acid moiety $n = 2$ and the compound was thus identified as 2*Z*,4*E*-octadienoic acid; this was confirmed by the presence in its mass spectrum of the fragment *m/z* 123 $[C_8H_{11}O]^+$ [6]. On comparing the ^{13}C NMR spectrum of **2** with the spectrum of phorbol [7], it was seen that the signal corresponding to C-4 was absent (75.30, *s*) whereas a methine group appeared at δ42.55 (*d*) suggesting that **2** was a 4β-deoxyphorbol.

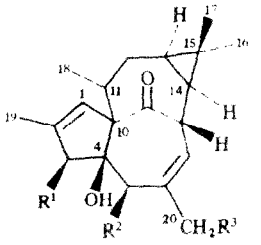
Partial hydrolysis of **2** (MeONa/MeOH) gave **2a** and **2b**. The ^{1}H NMR spectrum of **2a** (Table 3) showed the presence of a single acetoxyl group (δ2.12, 3H, *s*). The signal of the protons at C-20 appeared at δ4.0 which suggested that one of the acetoxyl groups must be situated at C-20. The ^{1}H NMR data also showed that **2a** was made up of a mixture of 4α and 4β epimers formed on hydrolysis of compound **2** [8].

The ^{1}H NMR spectrum of **2b** contained no acetoxyl signal and the signals of the H_2-20 and H-12 were shielded at δ4.00 and δ5.02, respectively, which showed that the hydroxyl groups at C-20 and C-13 were free [9] while in **2** they were acetylated. **2b** was made up of a mixture of epimers at C-4.

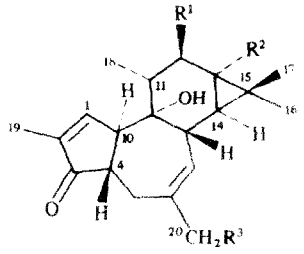
From these findings it was concluded that **2** was 12-*O*-(2*Z*,4*E*-octadienoyl)-4-deoxyphorbol-13,20-diacetate previously isolated from *E. biglandulosa* [6]. However, this is the first time that the complete and correct assignment of the signals of its ^{13}C NMR spectrum has been described (Table 2).

From the ^{1}H NMR and ^{13}C NMR spectra of **3** (Tables 3 and 4) and the data in the literature [7], it was concluded that **3** was a diterpene with a tigliane skeleton; more precisely a phorbol triester whose acid moieties were two molecules of acetic acid and one of 2*Z*-4*E*-octadienoic acid.

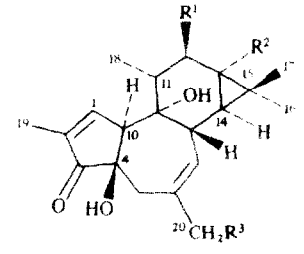
To determine the positions of the substituents, partial



	R^1	R^2	R^3
1	OCO—(CH=CH)$_2$—(CH$_2$)$_4$—Me	OH	OAc
4a	OCO—(CH$_2$)$_{12}$—Me	OAc	OAc
5a	OAc	OAc	OCO—(CH$_2$)$_{12}$—Me
6a	OAc	OCO—(CH$_2$)$_{12}$—Me	OCOMe



		R^1	R^2	R^3
2		OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OAc	OAc
2a	(4α,4β)	OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OAc	OH
2b	(4α,4β)	OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OH	OH



	R^1	R^2	R^3
3	OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OAc	OAc
3a	OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OAc	OH
3b	OCO—(CH=CH)$_2$—(CH$_2$)$_2$—Me	OH	OH

hydrolysis was performed to give **3a** and **3b**. By comparison of the ^{1}H NMR and ^{13}C NMR data (Tables 3 and 4) of these two compounds with those of compound **3**, the latter was assigned a structure of 13,20-*O*-diacetyl-12-*O*-(2*Z*,4*E*)-octadienoyl phorbol, a compound which has been isolated for the first time and whose spectroscopic properties coincide with those described by Hecker [10] for an acetylation product derived from *E. tirucalli L.*

The unequivocal assignment of all the signals of the ^{13}C NMR spectrum shown in Table 4 was performed by heteronuclear two-dimensional correlation (HCCORR). This is the model for the assignment of the signals of the ^{13}C NMR spectrum of compounds **1** and **2**.

The more polar hydroxyesters **4**, **5** and **6**, showed no signals for acetoxyl groups, and could only be separated from each other as their diacetyl derivatives (**4a**, **5a** and **6a**).

The ^{1}H NMR spectra (Table 1) showed signals of compounds with an ingenane skeleton: the olefinic hydrogens H-7 and H-1 at 6.23 (*d*) and 6.01 (*d*), respectively and the hydrogens geminal to the hydroxyl functions, which owing to their displacements to δ5.39 (*br s*, H-5) 4.95 (*s*, H-3) and 4.60 and 4.17 (*q* AB, 2H-20), established that the three hydroxyl groups, in all three compounds, were esterified, two of them by acetic acid and the other by a long chain, saturated fatty acid. GC of the methyl ester of the fatty acid released on hydrolysis showed that in each compound the acid substituent was myristic acid.

Table 1. ^{1}H NMR data for compounds with ingenane skeletons ($CDCl_3$)

H	1	4a	5a	6a
1	6.04 *q* (1.6)	6.08 *q* (1.2)	6.01 *q* (1.2)	6.08 *q* (1.4)
3	5.58 *s*	4.95 *s*	4.95 *s*	4.97 *s*
5	3.87 *br s*	5.41 *br s*	5.39 *br s*	5.38 *br s*
7	6.20 *br s*	6.23 *dd* (3.6; 0.9)	6.23 *d* (3.6)	6.23 *dd* (3.6; 0.9)
8	4.10 *dd* (11.5; 4.6)	4.28 *dd* (11.4; 4.5)	4.28 *dd* (11.5; 4.4)	4.24 *dd* (11.7; 4.4)
12*	2.50 *m*	2.82 *m*	2.52 *m*	2.51 *m*
13 14	0.72 *m*	0.72 *m*	0.72 *m*	0.72 *m*
Me-16	1.05 *s*	1.06 *s*	1.05 *s*	1.06 *s*
Me-17	1.07 *s*	1.09 *s*	1.07 *s*	1.08 *s*
Me-18	0.98 *d* (7.0)	0.99 *d* (7.3)	1.00 *d* (7.1)	0.99 *d* (7.3)
Me-19	1.80 *d* (1.6)	1.76 *d* (1.1)	1.75 *d* (1.2)	1.76 *d* (1.4)
H_2-20	4.48 *q*AB 4.76 $W_{1/2}$ = 12.5	4.52 *q*AB 4.20 $W_{1/2}$ = 12.5	4.60 *q*AB 4.17 $W_{1/2}$ = 12.5	4.58 *q*AB 4.20 $W_{1/2}$ = 12.6
2′	5.62 *d*	2.30* *m*	2.35* *m*	2.30* *m*
3′	6.64 *dd* (11.3; 11.3)	1.24* *br s*	1.24* *br s*	1.25* *br s*
4′	7.37 *ddddd* (15.1; 11.3; 1.3; 1.2; 1.3)	1.24* *br s*	1.24* *br s*	1.25* *br s*
5′	6.20 *m*	1.24* *br s*	1.24* *br s*	1.25* *br s*
H_2-6′	2.20 *m*	1.24 *br s*	1.24 *br s*	1.25 *br s*
10′	0.88† *t* (6.8)	1.24* *br s*	1.24* *br s*	1.25* *br s*
Me-14′	—	0.88 *t* (6.8)	0.87 *t* (6.7)	0.88 *t* (6.8)
OAc	2.6 *s*	2.12 *s* 1.99 *s*	2.22 *s* 1.99 *s*	2.22 *s* 2.13 *s*

The coupling constants (J, $W_{1/2}$) in parentheses are given in Hz.
*Intensity for two protons.
†Intensity for three protons.

The only difference between compounds **4a**, **5a** and **6a** is in the position of the fatty acid chain, as is clearly reflected in the different displacements at which the methyls of the acetoxyl groups appear in the ^{1}H NMR spectra ie **4a**: δ2.12 and 1.99; **5a**: 2.22 and 1.99 and **6a**: 2.22 and 2.13. Because it was not possible to carry out partial hydrolysis of these compounds owing to the occurrence of *trans*-esterification [11], the assignments of the positions of the substituents was performed according to the data appearing in the literature [3, 4, 12] **4a** was identified as 3-tetradecanoate-ingenol-5,20-diacetate; **5a** as 20-tetradecanoate-ingenol-3,5-diacetate and **6a** as 5-tetradecanoate-ingenol-3,20-diacetate.

Compounds **5** and **6** are described as natural products for the first time.

Confirmation of the ingenane skeleton of these compounds was performed by double irradiation experiments and by 2D COSY, and the unequivocal assignment of the signals of their ^{13}C NMR spectra, shown in Table 2, was conducted according to the relationships obtained from the ^{13}C–^{1}H (HCCORR) 2D correlation experiment carried out for compound **5a**.

EXPERIMENTAL

Mps: uncorr.; ^{1}H NMR: 200 MHz, TMS as int. standard; ^{13}C NMR: 50.3 MHz; EIMS: 70 eV (180°).

Extraction of the tetracyclic diterpenes from the latex. E. broteri (A herbarium sample of the plant is available from the Department of Botany, University of Salamanca.) was collected in flower in Béjar (Salamanca, Spain). The latex was squeezed from the fresh plant and collected in MeOH. The solid was sepd by filtration, extracted with Me_2CO at 40° and the triterpene compounds removed by partition with hexane [2]. The methanolic filtrate was evapd to dryness *in vacuo* (40°) and extracted with hexane (1.6 g, Fr. 1). The insoluble part (a yellow solid) was dissolved in MeOH–H_2O (1:1) and extracted with Et_2O (1.10 g, Fr. 2).

Fractions 1 and 2 showed the same composition by TLC. Fraction was chromatographed on silica gel (33 g) and seven fractions were collected (A–F). Fraction A (hexane–EtOAc, 9:1, 25.2%); fraction B (hexane–EtOAc, 4:1, 24.7%); fraction C (hexane–EtOAc, 4:1, 13%); fraction D (hexane–EtOAc, 1:1, 17.4%); fraction D (hexane–EtOAc, 1:1, 17.4%) and fraction F (hexane EtOAc, 1:1, 4.0%).

Table 2. ^{13}C NMR data for compounds with ingenane skeletons ($CDCl_3$)

C	1	4a	5a	6a		1	4a	5a	6a
1	132.20	132.28	132.20	132.34	C-1′	166.81		175.35	173.44
2	136.08		133.35	133.46	C-2′	114.36	33.97	34.66	34.18
3	82.52	82.37	82.08	82.35	C-3′	147.16	24.54	25.23	24.81
4	85.05		85.96	85.91	C-4′	127.03	29.82*	29.69*	29.71*
5	74.99	75.04	75.04	75.14	C-5′	147.16	29.74*	29.69*	29.71*
6	136.17		135.65	135.50	C-6′	33.07	29.74*	29.54*	29.51*
7	129.35	131.60	131.84	131.67	C-7′	28.49	29.53*	29.54*	29.51*
8	43.75	43.73	43.74	43.72	C-8′	31.46	29.53*	29.54*	29.34*
9	206.1		205.38	205.27	C-9′	22.46	29.40*	29.34*	29.34*
10	72.25		72.05	72.07	C-10′	13.94	29.25*	29.11*	29.17*
11	38.55	38.63	38.72	38.65	C-11′		29.53*	29.54*	29.51*
12	31.30	31.24	31.14	31.24	C-12′		31.98	31.95	31.95
13	23.43	23.08	23.06	23.09	C-13′		22.73	22.71	22.72
14	23.25	23.27	23.27	23.27	C-14′		14.14	14.11	14.13
								171.20	172.42
15	24.02		24.37	24.36	COMe	170.88		170.82	170.70
16	28.54	28.46	28.45	28.47	COMe	21.15	21.02	20.91	21.15
							20.80	20.82	20.80
17	15.48	15.40	15.38	15.37					
18	17.30	17.13	17.08	17.10					
19	15.53	15.62	15.56	15.57					
20	66.72	65.94	65.90	65.59					

*Assignment may be interchanged.

Isolation of diterpenes. Fraction B: CC on silica gel followed by prep. TLC (hexane–EtOAc, 7:3, twice) gave 35 mg **1**. Fraction C: Prep. TLC (hexane–EtOAc, 7:3, twice) gave 44 mg of compound **2**. Fraction E: CC on silica gel (6 g.) and eluting with hexane–EtOAc (4:1), gave **3** (0.106 g) and a mixture of **4** and **5** (0.074 g). The mixture was acetylated with Ac_2O–C_5H_5N and after purification by prep. TLC (C_6H_6–Et_2O, 4:1, twice), 8 mg **4** and 25 mg **5** were isolated. Fraction F: acetylation with Ac_2O–C_5H_5N and purification by prep. TLC (C_6H_6–Et_2O, 4:1, twice) gave 25 mg **5** and 11 mg **6**.

20-Acetyl-ingenol-3-decadienoate (**1**). Colourless oil. $[\alpha]_D^{25}$ −3.7° ($CHCl_3$; *c* 1.3); UV λ_{max}^{MeOH} nm (ε): 217 (13 700), 261 (23 230); IR ν_{max}^{film} cm^{-1}: 3700, 1730, 1650, 1610, 1200, 1160, 995, 990; ^1H NMR see Table 1; ^{13}C NMR see Table 2; MS (NH_3) *m/z* (rel. int.): 558 [M + NH_4]$^+$ (11.4), 541 (2), 522 (1.5), 506 (5), 490 (2), 480 (1.5), 438 (1.5), 408 (32.3), 390 (19), 374 (7), 356 (3), 355 (3), 330 (22), 314 (6), 312 (13.2), 294 (4.5), 272 (4.4), 202 (100), 184 (13.2).

Alkaline hydrolysis of **1**. Compound **1** (30 mg) was treated with 0.5 M KOH in dry MeOH at room temp. for 20 min. The MeOH was removed by evapn and the aq. phase extracted with $CHCl_3$ to give 8 mg of a product, which was esterified with an excess of ethereal soln of CH_2N_2 and identified as *2Z,4E-decadienoic methyl ester* (**1a**). IR ν_{max}^{film} cm^{-1}: 1730, 1650, 1610, 1160, 1000 and 970; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.36 (1H, *ddddd*, *J* = 15.1, 11.7, 1.3, 1.3, 1.2 Hz, H-4′), 6.56 (1H, *dd*, *J* = 11.7, H-3′), 6.09 (1H, *ddd*, *J* = 14.6, 6.8 Hz, H-5′), 5.57 (1H, *d*, *J* = 11.7 Hz, H-2′), 3.72 (3H, *s*, –COOMe), 1.25 [*br s*, –$(CH_2)_n$–], 0.89 (3H, *t*, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz): 174.30 (*s*, C-1), 111.15 (*d*, C-2), 145.76 (*d*, C-3), 127.03 (*d*, C-4), 145.44 (*d*, C-5), 32.92 (*t*, C-6), 28.50 (*t*, C-7), 31.44 (*t*, C-8), 22.43 (*t*, C-9), 13.87 (*q*, C-10).

Hydrogenation of **1a**. **1a** (8 mg) in Et_2O was hydrogenated [PtO_2 (2 mg)/H_2] overnight at 4°. The product recovered from the reaction mixture was identified as methyl caproate by GC analysis (DEGS, 10%, 2 m, 118°, 130).

12-O-(2Z,4E-Octadienoyl)-4-deoxyphorbol-13,20-diacetate (**2**). Colourless oil. $[\alpha]_D^{25}$ 0° ($CHCl_3$; *c* 1.64); UV λ_{max}^{MeOH} nm (ε): 220 (8380), 261 (26760), 310 (140); IR ν_{max}^{film} cm^{-1}: 3420, 1750, 1730, 1660, 1620, 995; ^1H NMR: see Table 3; ^{13}C NMR: see Table 4; MS *m/z* (rel. int.) 554 (0.1), 536 (0.1), 494 (1.5), 434 (2.0), 415 (2.0), 414 (1.35), 355 (9.2), 354 (6.3), 313 (6.3), 312 (11.0), 295 (12.1), 294 (26.7), 123 (100).

Partial hydrolysis of **2**. To a soln of **2** (75 mg) in absolute MeOH (15 ml) was added (0.5 g/100 ml) NaOMe/MeOH (3 ml) and the soln left to stand at 4° for 48 hr. The reaction mixture was worked up by adding a few drops of HOAc, evapg the MeOH and extracting the aq. phase with $CHCl_3$ to give 55 mg of crude product. This was chromatographed on silica gel (28 g) eluted with $CHCl_3$–MeOH (4:1 and 2:1) to give **2a** and **2b** respectively.

12-O-(2Z,4E-Octadienoyl)-(4α,4β)-desoxyphorbol-13-acetate (**2a**). IR ν_{max} cm^{-1}: 3400, 3000, 2940, 2920, 2860, 1720, 1705, 1645, 1600, 1250 and 970; ^1H NMR ($CDCl_3$, 200 MHz): see Table 3.

12-O-(2Z,4E-Octadienoyl)-(4α,4β)-deoxyphorbol (**2b**). IR ν_{max} cm^{-1}: 3400, 2920, 3010, 1720, 1640, 1610, 1150, and 975; ^1H NMR ($CDCl_3$, 200 MHz): see Table 3.

12-O-(2Z,4E-Octadienoyl)-phorbol-13,20-diacetate (**3**). $[\alpha]_D$ +24° ($CHCl_3$, *c* 1.6); UV λ_{max}^{MeOH} nm (ε): 230 (12.630), 260 (23.230), 310 (140); IR ν_{max}^{film} cm^{-1}: 3420, 1730, 1710, 1640, 1605, 995, 965; ^1H NMR: see Table 3; ^{13}C NMR: see Table 4; MS *m/z* (rel. int.): 431 (0.27), 370 (1.22), 371 (0.61), 329 (0.76), 328 (2.12), 312 (1.2), 311 (1.6), 310 (3.4), 293 (1.4), 292 (1.6), 295 (1.7), 123 (12).

Partial hydrolysis of **3**. To a soln of compound **3** (80 mg) in absolute MeOH (15 ml) was added (0.5 g/100 ml) NaOMe/MeOH (3 ml). After 48 hr the product was recovered in the usual way yielding a mixture (54 mg) of two compounds (**3a** and **3b**). The mixture was chromatographed on silica gel affording **3a** (17 mg, $CHCl_3$–Me_2CO, 4:1) and **3b** (22 mg, $CHCl_3$–Me_2CO, 2:1).

12-O-(2Z,4E-Octadienoyl)-phorbol-13-acetate (**3a**).

Table 3. ^{1}H NMR data for compounds with tigliane skeletons ($CDCl_3$)

H	2	2a	2b	3	3a	3b
1	7.55 *br s*	7.55 *br s* 7.05 *br s*	7.55 *br s*	7.57 *br s*	7.57 *br s*	7.57 *br*
4	2.60 *m*	2.60 *m* 3.00 *m*	2.60 *m* 3.00 *m*	—	—	—
H_2-5	2.84 *m*	2.84 *m* 5.60 *d* (5.1)	2.84 *m* 5.60 *d* (5.1)	a 2.55 *m* b 2.37 *m*	2.65 *m*	2.65 *m*
7	5.60 *d* (7.0)	5.13 *d* (5.2)	5.13 *d* (5.2)	5.68 *dd* (5.3; 1.4)	5.68 *d* (5.4)	5.68 *d* (5.4)
8	2.45 *m*	2.45 *m*	2.45 *m*	3.20 *m*	3.20 *m*	3.18 *m*
10	3.24 *br s*	3.24 *m* 3.51 *m*	3.24 *m* 3.51 *m*	3.11 *m*	3.20 *m*	3.18 *m*
12	5.44 *d* (9.7)	5.44 *d* (10.0)	5.07 *d* (10.1)	5.42 *d* (10.2)	5.42 *d* (10.2)	4.85 *d* (10.2)
14	1.03 *d* (5.3)	1.03 *d* (5.3)	1.11 *d* (5.3)	1.03 *d* (4.8)	1.03 *d* (4.8)	1.11 *d* (4.8)
Me-16	1.19 *s*	1.19 *s*	1.19 *s*	1.17 *s*	1.17 *s*	1.17 *s*
Me-17	1.20 *s*	1.20 *s*	1.20 *s*	1.21 *s*	1.21 *s*	1.21 *s*
Me-18	0.92 *d* (6.6)	0.92 *d* (6.5)	0.92 *d* (6.5)	0.86 *d* (6.1)	0.87 *d* (6.0)	0.86 *d* (6.0)
Me-19	1.72 *dd* (2.4; 1.2)	1.72 *d* (2.9)	1.72 *d* (2.9)	1.71 *dd* (2.9; 1.4)	1.71 *dd* (2.9; 1.4)	1.75 *dd* (2.9; 1.4)
H_2-20	4.44 *s*	4.00 *s* 3.95 *s*	4.00 *s* 3.95 *s*	4.42 *s*	4.00 *s*	4.00 *s*
2′	5.51 *d* (11.3)	5.51 *d* (11.3)	5.50 *d* (11.3)	5.51 *d* (11.3)	5.51 *d* (11.2)	5.51 *d* (11.2)
3′	6.58 *dd* (11.3; 11.3)	6.58 *t* (11.3)	6.58 *t* (11.3)	6.54 *dd* (11.3; 11.3)	6.54 *t* (11.2)	6.54 *t* (11.2)
4′	7.33 *ddddd* (15.1; 11.3; 1.3; 1.3; 1.2)	7.33 *dd* (15.1; 11.3)	7.33 *dd* (15.1; 11.3)	7.30 *ddddd* (15.1; 11.2; 1.3; 1.3; 1.2)	7.30 *dd* (15.1; 11.2)	7.30 *dd* (15.1; 11.2)
5′	6.09 *ddd* (15.1; 7.3; 7.3)	6.09 *dt* (15.1; 11.3)	6.09 *dt* (15.1; 7.3)	6.05 *dt* (15.1; 7.3)	6.05 *dt* (15.1; 7.3)	6.05 *dt* (15.1; 7.3)
H_2-6′	2.20 *m*	2.20 *m*	2.20 *m*	2.17 *m*	2.17 *m*	2.17 *m*
Me-8′	0.92 *t* (7.0)	0.92 *t* (7.2)	0.92 *t* (7.2)	0.86 *t* (7.3)	0.87 *t* (6.8)	0.87 *t* (6.8)
OAc	2.11 *s* 2.05 *s*	2.12 *s*		2.08 *s* 2.01 *s*	2.10 *s*	

IR ν_{max}^{film} cm^{-1}: 3420, 1730, 1710, 1640, 1605, 1250, 995 and 965; ^{1}H NMR: see Table 3; ^{13}C NMR: see Table 4.

12-O-(2Z,4E-Octadienoyl)-phorbol (**3b**). IR ν_{max}cm^{-1}: 3420, 1730, 1710, 1640, 1605, 995; ^{1}H NMR: see Table 3; ^{13}C NMR: see Table 4.

3-*Tetradecanoate-ingenol*-5,20-*diacetate* (**4a**). $[\alpha]_D$ −4° ($CHCl_3$, *c* 0.3); UV λ_{max}^{MeOH} nm: 220 (13.700), 260 (230); IR ν_{max} cm^{-1}: 3440, 1750, 1640; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; MS *m/z* (rel. int.): 582 (0.55), 522 (1.5), 521 (1.0), 493 (0.9), 414 (1.1), 372 (3.8), 355 (3.2), 354 (2.7), 337 (1.1), 330 (1.3), 327 (1.2), 326 (1.9), 313 (9.6), 312 (13.2), 295 (9.3), 294 (13.8), 284 (11.7), 266 (13.2), 251 (22.5), 223 (23), 133 (18), 121 (35).

Alkaline hydrolysis of **4a**. **4a** (8 mg) was hydrolysed with 0.5 M NaOH in MeOH (0.8 ml) at room temp. for 20 min. After usual work-up the product was extracted (2 mg) esterified with CH_2N_2 and identified as methyl myristate by GC.

20-*Tetradecanoate-ingenol*-3,5-*diacetate* (**5a**). Oil. $[\alpha]_D$ +5.7° ($CHCl_3$, *c* 2.16); UV λ_{max}^{MeOH} nm (ε): 221 (13.700), 262 (220); IR ν_{max}^{film} cm^{-1}: 3420, 1740, 1730, 1640; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; MS *m/z* (rel. int.): 569 (0.27), 522 (0.37), 485 (1.05), 414 (1.66), 373 (1.03), 372 (2.83), 371 (1.34), 355 (4.8), 354 (6.6), 330 (1.5), 313 (14.5), 312 (20.9), 294 (19), 223 (23), 121 (52).

Alkaline hydrolysis of **5a**. (40 mg) was performed as for **4a**. The product (28 mg) after purification by CC (hexane–EtOAc, 9:1) gave 8 mg) methyl myristate (identified by GC).

5-*Tetradecanoate-ingenol*-3,20-*diacetate* (**6a**). Colourless oil. $[\alpha]_D$ −1° ($CHCl_3$; *c* 0.4); UV λ_{max}^{MeOH} nm (ε): 229 (13.700), 278 (230); IR ν_{max}^{film} cm^{-1}: 3440, 1745, 1735, 1640; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; MS *m/z* (rel. int.): $[M]^+$ absent, 533 (3.6), 510 (1.0), 509 (3.7), 481 (2.3), 373 (1.55), 372 (3.8), 354 (5.5), 337

Table 4. ^{13}C NMR data of compounds with tigliane skeletons ($CDCl_3$)

C	2	3	3a	3b		2	3	3a	3b
1	159.66	160.84	160.72	160.30	C-1′	166.06	166.26	166.19	166.20
2	137.31	135.68	140.54	140.93	C-2′	114.85	115.21	115.34	114.77
3	208.85	208.55	208.85	208.85	C-3′	146.24	145.83	145.68	146.60
4	42.55	73.61	73.84	73.61	C-4′	127.13	127.20	127.27	127.03
5	35.07	38.83	38.72	38.75	C-5′	146.17	145.75	145.58	146.45
6	136.46	132.88	132.86	133.43	C-6′	30.04	35.02	35.01	35.04
7	130.23	132.71	129.32	129.40	C-7′	22.05	21.99	22.00	21.90
8	42.29	39.36	39.24	39.16	C-8′	13.72	13.71	13.66	13.67
9	77.76	78.18	78.27	79.18	COMe	20.97 21.13	20.91 21.13	21.10	
10	54.13	56.17	56.32	57.04	COMe	173.11 170.68	173.77 170.74	170.72	
11	44.14	43.18	43.27	43.53					
12	76.09	76.09	77.21	87.32					
13	65.36	65.68	65.68	61.03					
14	35.38	36.09	36.34	35.29					
15	25.68	25.70	25.70	27.69					
16	23.75	23.83	23.84	22.36					
17	16.66	16.74	16.75	17.02					
18	15.09	14.38	14.38	15.35					
19	10.18	10.06	10.01	10.07					
20	68.88	69.38	68.04	67.96					

(1.2), 313 (10.2), 312 (21), 295 (14), 294 (20), 121 (35).

Alkaline hydrolysis of **6a** (10 mg) was performed as for **4a**. The product was methyl myristate (GC).

REFERENCES

1. Evans, F. J. and Soper, C. J. (1978) *J. Nat. Prod.* **41**, 193.
2. Pascual Teresa, J. de, Urones, J. G., Basabe, P., Marcos, I. S., Sexmero, M^a J. and Fernandez Moro, R. (1987) *Phytochemistry* **26**, 1767.
3. Abo, K. and Evans, F. J. (1982) *Phytochemistry* **21**, 725.
4. Zechmeister, K., Hecker, E. and Adolf, W. (1970) *Tetrahedron Letters* 4075.
5. Unemura, D., Ohwaki, H. and Hirata, Y. (1974) *Tetrahedron Letters* **25**, 2527.
6. Falsone, G. and Crea, A. F. G. (1979) *Liebigs Ann. Chem.* 1116.
7. Neeman, M. and Simmons, O. D. (1979) *Can. J. Chem.* **57**, 2071.
8. Furstenberger, G. and Hecker, E. (1972) *Tetrahedron Letters* 925.
9. Szczepanski, CH. V. and Hecker, E. (1967) *Liebigs Ann. Chem.* **705**, 199.
10. Furstenberger, G. and Hecker, E. (1977) *Experientia* **33**, 986.
11. Abo, K. and Evans, F. J. (1982) *J. Nat. Prod.* **45**, 365.
12. Evans, F. J. and Kinghorn, A. D. (1974) *Phytochemistry* **13**, 2324.