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INGENANE AND LATHYRANE DITERPENES FROM THE LATEX OF *EUPHORBIA ACRURENSIS*

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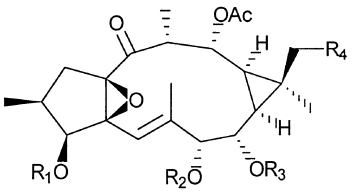
Key Word Index—*Euphorbia acrurensis*; Euphorbiaceae; diterpenes; ingenanes; lathyranes; ingenol esters; ingol esters; triterpenes.

Abstract—The latex of *Euphorbia acrurensis* yielded, in addition to the widespread triterpenes euphol and euphorbol as the major components, two ingenol esters and nine lathyrane derivatives as minor components. All the lathyrane diterpenes were esters of ingol, and five of them were new. The structures were established with the aid of spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved

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 [1, 2]. In recent treatments of its scope [2], 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 latex [3–6]. 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 [6, 7]. Further diterpenes belonging to other skeletal types, *e.g.* ingenane derivatives, are also characterized by similarly strong pharmacological effects [6].



1 R_1 = Ac R_2 = R_3 = Tigl R_4 = OH
2 R_1 = Ac R_2 = H R_3 = Tigl R_4 = OAc
3 R_1 = R_2 = H R_3 = Tigl R_4 = OAc
4 R_1 = R_3 = Ac R_2 = iVal R_4 = H
5 R_1 = R_3 = Ac R_2 = Ang R_4 = H
6 R_1 = Ac R_2 = R_3 = Tigl R_4 = H
7 R_1 = R_3 = Ac R_2 = Tigl R_4 = H
8 R_1 = Ac R_2 = Tigl R_3 = Me R_4 = H
9 R_1 = Ac R_2 = H R_3 = Tigl R_4 = H

RESULTS AND DISCUSSION

In the context of our recent interest in the chemistry of the genus *Euphorbia* [8–11], we have now investigated the chemical constituents of *E. acrurensis* N. E. Brown (syn. *E. abyssinica* G. A. Schweinfurth) [12]. The irritant latex of this species consisted mainly (≈90% of dry weight) of two triterpenes, euphol and euphorbol, widespread in the genus [5]. We further isolated as minor components the known ingenol esters **10** [10] and **11**† [13, 14] and the ingol esters **1**–**9**. The structures of these compounds were established through extensive 1D (spin decoupling, NOE) and 2D (HMQC, HMBC) NMR measurements. The latter were needed for both the establishment of the carbon framework and for the location of the different types of ester residues [8–11]. Compounds **1**–**9** were thus identified as ingol esters bearing various types of acyl groups, acetyl and tigloyl moieties being most frequent. The configurations at the stereogenic centres were deduced from coupling constants values, NOE measurements and comparison with literature data. In all cases, the values of $J_{2,3}$ (≈8.5 Hz) and the chemical shifts of H-1α and H-1β (Table 1) indicated that the configurations at C-2 and C-3 are as in true ingol derivatives [10].

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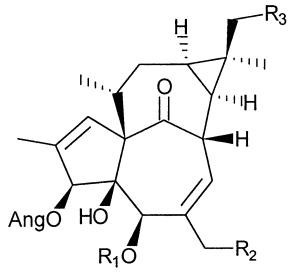
† Compound **11** is depicted in Ref. [14] with the wrong configuration at C-15 (oxygen function at C-16 instead of C-17).



10 R_1 = Ac R_2 = OAc R_3 = H
11 R_1 = R_2 = H R_3 = OAc

Compounds **1**–**3** belong to the rare class of lathyrane derivatives functionalized in the *gem*-dimethylcyclopropane moiety [15–20]. This was deduced from the absence of one of the two characteristic gem-dimethyl singlets, new signals in the range δ 3–4.5 being now visible, either as doublets or as double doublets (Table 1). These signals showed marked NOEs with H-8 and H-12 but not with H-9 and H-11. This settled the configuration at C-10 and located the oxygen function at C-19. For all compounds, HMBC correlations between the signals from H-3/H-12 and carbonyl acetate peaks allowed us to place acetyl groups on the corresponding hydroxyl atoms (except for **3**, which has a free OH group at C-3). In a similar way, the other acyl groups were assigned to their corresponding oxygen atoms. Seven out of the nine ingol esters (**1**–**3**, **6**–**9**) display at least one tigloyloxy group. In six of them this group is located at C-8. Only in compound **7** is the single tiglate bound to C-7, whereas in two compounds (**1**, **6**) two tiglate esters are present at both C-7 and C-8. Compound **5** is the only isolated ingol ester having an angelate residue. It was found as a minor component (ca 20%) in admixture with **4** and resisted tenaciously all attempts at separation. Its NMR signals had to be sorted out of those of the major compound and its structure assignment therefore is only tentative. However, comparison with the published ^{1}H NMR and ^{13}C NMR data of ingol 3,8,12-triacetate 7-angelate supports the correctness of our proposal [21, 22].

Compounds **1**–**4** and possibly **6** have not been previously described in the literature. With the other four compounds, the situation is not always clear-cut. Compounds **8** [15, 22] and **9** [23] have been described with detail, including ^{1}H NMR and ^{13}C NMR data. Positional isomers of **7** (ingol 3,8,12-triacetate 7-tiglate), as regards the location of the acyl groups, have been reported [15, 24] but some of the structural assignments were based on the unreliable method of partial hydrolysis [22] and possibly may not be correct. For instance, the ^{1}H NMR data of a compound claimed to be ingol 3,7,8-triacetate 12-tiglate [24] are too close to those of **7** to exclude their identity. Furthermore, a mixture of ingol esters from *E. kamerunica* has been reported to contain ingol 3,7,12-triacetate 8-angelate and the corresponding 8-tiglate [21] but it was later suggested that the compounds were actually the 7-angelate and 7-tiglate isomers (i.e. **5** and **7**) [22]. An ingol triacetate monotiglate and a diacetate ditiglate have been reported in *E. ledienii* but the precise locations of the acyl moieties were not ascertained [25] and almost no NMR data were given. For these reasons and in order to avoid future erroneous assignments, we give in Tables 1 and 2 the complete high resolution ^{1}H NMR and ^{13}C NMR spectral data of compounds **6** and **7**, together with those of **1**–**4**. Ingol 3,7,12-triacetate 8-isovalerate, a positional isomer of **4**, has been previously reported [26].

EXPERIMENTAL

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) 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.

Plant material

The latex of *E. acrurensis* (ca 120 g) was collected from plant specimens growing in the Botanical Acclimatization Garden at La Orotava, Teneriffa, Canary Islands, in May 1996.

Extraction and chromatography

Dissolution of the latex in hot MeOH (1.2 l) and re-cooling to room temp. gave rise to a voluminous, white ppt. (ca 7.5 g) of the widespread triterpenes euphol and euphorbol, which was eliminated by filtration. Evaporation of the solvent *in vacuo* gave a whitish, oily material (ca 17.5 g). After dissolving this oil in the minimum amount of MeOH, reverse-phase silica gel was added (3 g of silica gel/g of extract) [8–11]. 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 H_2O, then with MeOH–H_2O (70:30) and finally with MeOH. The H_2O and MeOH fractions only contained polar, ill-defined compounds and common triterpenes (mainly euphol and euphorbol), 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 (560 mg) which was then subjected to further chromatographic separations as described below.

The middle fraction was subjected to CC on silica gel (elution with hexane–Et_2O 10:1 → Et_2O). The intermediate fractions were further purified, where

Table 1. ^{1}H NMR spectral data of ingol derivatives **1**–**4**, **6** and **7**

H	**1**	**2**	**3**	**4**	**6**	**7**
H-1α	1.69 *d* (15)	1.65 *d* (15)	1.65 *d* (15)	1.68 *d* (15)	1.68 *d* (15)	1.67 *d* (15)
H-1β	2.79 *dd* (15; 9)	2.81 *dd* (15; 9)	2.76 *dd* (15; 9)	2.78 *dd* (15; 9)	2.78 *dd* (15; 9)	2.77 *dd* (15; 9)
H-2	2.51 *m*	2.56 *m*	2.50 *m*	2.51 *m*	2.49 *m*	2.48 *m*
H-3	5.21 *d* (8.5)	5.20 *d* (8.3)	4.36 *d* (8.5)	5.19 *d* (8.5)	5.22 *d* (8.5)	5.20 *d* (8.5)
H-5	5.56 *br s*	5.78 *br s*	5.85 *br s*	5.58 *br s*	5.56 *br s*	5.55 *br s*
H-7	5.24 *br s*	4.29 *br s*	4.29 *br s*	5.20 *br s*	5.20 *d* (2)	5.20 *br s*
H-8	4.70 *dd* (10.5; 2.5)	4.52 *dd* (10.5; 1.5)	4.54 *dd* (10.7; 1.5)	4.57 *dd* (10.7; 2)	4.66 *dd* (10.7; 2)	4.58 *dd* (10.7; 2)
H-9	1.43 *dd* (10.5; 9)	1.65 *dd* (10.5; 9)	1.65 *dd* (10.7; 9)	1.26 *dd* (10.7; 9)	1.32 *dd* (10.7; 9)	1.25 *dd* (10.7; 9)
H-11	1.31 *dd* (11; 9)	1.33 *dd* (11; 9)	1.31 *dd* (11; 9)	1.12 *dd* (11; 9)	1.14 *dd* (11; 9)	1.12 *dd* (11; 9)
H-12	5.00 *dd* (11; 4)	4.92 *dd* (11; 4)	4.92 *dd* (11; 4)	4.86 *dd* (11; 4)	4.89 *dd* (11; 4)	4.86 *dd* (11; 4)
H-13	2.94 *dq* (4; 7)	2.88 *dq* (4; 7)	2.89 *dq* (4; 7)	2.92 *dq* (4; 7)	2.95 *dq* (4; 7)	2.92 *dq* (4; 7)
H-16	0.92 *d* (7.5)	0.89 *d* (7.5)	1.03 (*d* (7.5)	0.91 *d* (7.5)	0.91 *d* (7.5)	0.90 *d* (7.5)
H-17	2.12 *br s**	2.05 *d* (1)	2.05 *d* (1)	2.10 *d* (1)	2.13 *d* (1)	2.10 *br s*
H-18	1.19 *s*	1.11 *s*	1.11 *s*	1.08 *s*	1.09 *s*	1.08 *s*
H-19	3.52 *dd* (12; 5.5) 3.35 *dd* (12; 6)	4.20 *d* (12) 3.54 *d* (12)	4.20 *d* (12) 3.54 *d* (12)	0.85 *s*	0.81 *s*	0.84 *s*
H-20	1.10 *d* (7)	1.07 *d* (7)	1.05 *d* (7)	1.06 *d* (7)	1.07 *d* (7)	1.06 *d* (7)
OAc	2.11 *s*, 2.07 *s*	2.10 *s*, 2.09 *s* 1.93 *s*	2.09 *s*, 1.93 *s*	2.09 *s*, 2.06 *s* 2.00 *s*	2.09 *s*, 2.07 *s*	2.08 *s*, 2.06 *s* 1.99 *s*
OCOR	6.85 *qq* (7; 1.5) 6.80 *qq* (7; 1.5) 1.84 *d* (1.5) 1.83 *d* (1.5) 1.81 *dq* (7; 1.5) 1.79 *dq* (7; 1.5)	6.91 *qq* (7; 1.5) 1.84 *d* (1.5) 1.81 *dq* (7; 1.5)	6.90 *qq* (7; 1.5) 1.84 *d* (1.5) 1.80 *dq* (7; 1.5)	2.27 *d* (7) 2.10 *m** 0.94 *d* (6.5) 0.93 *d* (6.5)	6.85 *qq* (7; 1.5) 6.80 *qq* (7; 1.5) 1.84 *d* (1.5) 1.83 *d* (1.5) 1.81 *dq* (7; 1.5) 1.79 *dq* (7; 1.5)	6.87 *qq* (7; 1.5) 1.84 *d* (1.5) 1.81 *dq* (7; 1.5)

δ in ppm and *J* (parentheses) in Hz (400 MHz, $CDCl_3$, 22°C).
* Overlapped signal.

Table 2. ^{13}C NMR spectral data of ingol derivatives **1**–**4**, **6** and **7**

C	**1**	**2**	**3**	**4**	**6**	**7**
C-1	31.7	31.7	31.8	31.5	31.5	31.5
C-2	29.6	29.7	31.7	29.5	29.5	29.5
C-3	76.7	77.6	76.3	76.9	76.7	76.7
C-4	73.4	73.7	76.0	73.3	73.4	73.4
C-5	116.5	116.5	117.0	117.2	116.5	116.7
C-6	140.2	141.5	141.7	139.7	140.2	139.8
C-7	76.4	76.0	75.9	76.2	76.7	76.5
C-8	71.3	73.6	73.4	71.5	71.6	71.6
C-9	24.9	23.8	23.9	24.7	25.2	24.9
C-10	25.3	22.0	21.9	19.3	19.3	19.3
C-11	31.1	31.2	31.3	30.7	30.8	30.7
C-12	70.5	69.4	69.5	70.6	70.7	70.6
C-13	43.6	43.5	43.3	43.1	43.2	43.1
C-14	207.2	207.4	207.6	207.6	207.7	207.6
C-15	71.2	71.5	72.7	71.1	71.1	71.1
C-16	17.0	17.0	16.1	16.9	17.0	17.0
C-17	17.4	17.4	17.5	17.6	17.5	17.5
C-18	24.5	24.4	24.4	29.0	29.3	29.2
C-19	62.6	64.9	64.8	16.1	16.1	16.1
C-20	13.3	13.4	13.4	13.4	13.4	13.4
OAc	172.2, 170.6	170.7, 170.6, 170.3	170.6, 170.4	170.5, 170.4, 170.3	170.6, 170.4	170.6, 170.4, 170.3
	21.2, 20.6	21.2, 20.7, 20.6	21.1, 20.6	21.0, 20.9, 20.5	21.0, 20.6	21.0 (×2), 20.6
OCOR	166.8, 166.4	167.3	167.3	171.8 (C═O)	167.1, 166.5	166.6
	138.5, 138.0	138.9	138.8	43.5 (CH_2)	137.9, 137.7	137.9
	128.4, 128.1	128.2	128.2	26.0 (CH)	128.5, 128.2	128.5
	14.6, 14.5	14.6	14.6	22.2 (CH_3)	14.5, 14.4	14.6
	12.2, 12.0	12.1	12.1	22.1 (CH_3)	12.2, 11.9	12.2

δ in ppm (100 MHz, $CDCl_3$, 22°C). Signals have been assigned by means of 2D NMR experiments.

necessary, by prep. TLC and/or HPLC. This allowed the isolation of additional amounts of triterpenes and of the compounds mentioned in the text. The latter were eluted from the silica gel column in the following order of increasing polarity: **4**/**5** (2 mg), **10** (2 mg), **6** (2 mg), **8** (1 mg), **7** (30 mg), **11** (2 mg), **9** (10 mg), **1** (15 mg), **2** (10 mg) and **3** (25 mg).

19-*Hydroxyingol* 3,12-*diacetate* 7,8-*ditiglate* (**1**). Oil, $[\alpha]_D$ −29° ($CHCl_3$; *c* 4.8); IR ν_{max}^{film} cm^{-1}: 1720 (*br*, ester and ketone C═O); EIMS (probe) *m*/*z* (rel. int.): 630.3031 $[M]^+$ (24), 570 $[M–AcOH]^+$ (9), 530 $[M–C_5H_8O_2]^+$ (100), 388 $[M–2C_5H_8O_2–CH_2{=}C{=}O]^+$ (79). Calc. for $C_{34}H_{46}O_{11}$, *M* = 630.3040; NMR, Tables 1 and 2.

19-*Hydroxyingol* 3,12,19-*triacetate* 8-*tiglate* (**2**). Oil, $[\alpha]_D$ −15° ($CHCl_3$; *c* 1); IR ν_{max}^{film} cm^{-1}: 1725 (*br*, ester and ketone C═O); EIMS (probe) *m*/*z* (rel. int.): 590.2720 $[M]^+$ (4), 530 $[M–AcOH]^+$ (14), 430 $[M–C_5H_8O_2–AcOH]^+$ (6), 388 $[M–C_5H_8O_2–AcOH–CH_2{=}C{=}O]^+$ (15). Calc. for $C_{31}H_{42}O_{11}$, *M* = 590.2727; NMR, Tables 1 and 2.

19-*Hydroxyingol* 12,19-*diacetate* 8-*tiglate* (**3**). Oil, $[\alpha]_D$ +2 ($CHCl_3$; *c* 4); IR ν_{max}^{film} cm^{-1}: 1725 (*br*, ester and ketone C═O); EIMS (probe) *m*/*z* (rel. int.): 548.2626 $[M]^+$ (55), 530 $[M–H_2O]^+$ (50), 488 $[M–AcOH]^+$ (16), 448 $[M–C_5H_8O_2]^+$ (16), 388 $[M–C_5H_8O_2–AcOH]^+$ (65). Calc. for $C_{29}H_{40}O_{10}$, *M* = 548.2621; NMR, Tables 1 and 2.

Ingol 3,8,12-*triacetate* 8-*isovalerate* (**4**). Oil, containing ca 20% of **5**; IR ν_{max}^{film} cm^{-1}: 1730 (*br*, ester and ketone C═O); EIMS (probe) *m*/*z* (rel. int.): 576.2930 $[M]^+$ (55), 516 $[M–AcOH]^+$ (5), 492 $[M–C_3H_7CH{=}C{=}O]^+$ (10), 432 $[M–C_3H_7CH{=}C{=}O–AcOH]^+$ (33), 372 $[M–C_3H_7CH{=}C{=}O–2AcOH]^+$ (24). Calc. for $C_{31}H_{44}O_{10}$, *M* = 576.2934; NMR, Tables 1 and 2.

Ingol 3,12-*diacetate* 7,8-*ditiglate* (**6**). Oil; IR ν_{max}^{film} cm^{-1}: 1730 (*br*, ester and ketone C═O); EIMS (probe) *m*/*z* (rel. int.): 614.3079 $[M]^+$ (100), 554 $[M–AcOH]^+$ (5), 472 $[M–C_5H_8O_2–CH_2{=}C{=}O]^+$ (18), 372 $[M–2C_5H_8O_2–CH_2{=}C{=}O]^+$ (9), 330 $[M–2C_5H_8O_2–2CH_2{=}C{=}O]^+$ (25). Calc. for $C_{34}H_{46}O_{10}$, *M* = 614.3091; NMR, Tables 1 and 2.

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