Constituents of Egyptian Euphorbiaceae. IX. Irritant and cytotoxic ingenane esters from *Euphorbia paralias* L.

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Summary. The irritant and cytotoxic constituents of the latex of *Euphorbia paralias* L. were separated from the hydrocarbon fraction by means of solvent partition. 3 new ingenane esters were identified from the toxic ether fraction. The major compound was 3-angelyl-20-deoxyingenol and the 2 minor compounds were 3-hexanoyl-20-deoxyingenol and 3-angelyl-ingenol. These compounds were of a similar potency to podophyllin in the inhibition of ^{3}H-thymidine uptake by TLX/5 mouse lymphoma cells. In addition the compounds produced a persistent erythema of the mouse ear in sub-microgram doses.

Euphorbia paralias L. is a small glabrous plant which grows on the maritime sand dunes in the western Egyptian desert[3]. Interest has been shown in plants of this genus as sources of rubber and hydrocarbons[4], possibly as part of a desert reclamation programme. It has been shown that members of the *Euphorbia* are responsible for the production of erythema on both human and animal skin[5]. The biologically active compounds are diterpene esters[6]. The majority of plants so far investigated for diterpenes are large tree-like species indigenous to central and southern Africa[6].

By means of an erythema test[7] the acetone macerate of the dried fresh latex of *Euphorbia paralias*[3] was shown to induce pronounced inflammation of the mouse ear. The residue from this macerate was separated by solvent partition into a methanol/water, an ether and a hexane soluble fraction. Only the residue from the ether fraction was biologically active. This fraction demonstrated marked cytotoxic activity against TLX/5 mouse lymphoma cells[8] as well as inducing inflammation of the mouse ear. 3 biologically active compounds were isolated from the ether soluble residue by means of adsorption[9] and partition[10] preparative TLC-methods.

The major component of the mixture, **1**, was a clear glassy resin, R_f 0.38 (Silica gel G, 500-µm layers, solvent chloroform/ether (95/5); IR: γ_{max} (solid film KBr disc): 3430, 1740, 1705 and 1640 cm^{-1}; C.D. (Methanol):

200 $[\theta] = -8060$ 220 $[\theta] = +20493$

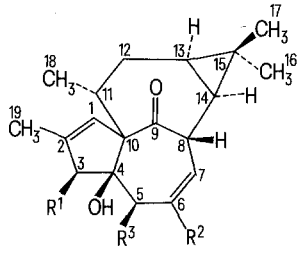
284 $[\theta] = -9290$ 309 nm $[\theta] = +2185$

m.s. (electron impact, 70 ev, 170°): exhibited no $M^{+\cdot}$ ion but major fragment ions were evident at m/z 314 (60%, $C_{20}H_{26}O_3$), 296 (40%) and 121 (100%). The base peak at m/z 121 is typical of ingenane diterpenes[11]; chemical ionisation m.s. (methane, 150°): exhibited $M^{+\cdot}$ ion at m/z 414 (4%) together with an $M^{+\cdot}+1$ ion at m/z 415 (20%) and fragment ions at m/z 315 (100%), 314 (20%), 297 (40%) and 296 (20%); NMR (60 MHz, $CDCl_3$, TMS = 0.00 ppm): δ 6.17 (m, 1H), 6.08 (bs, 1H), 5.79 (d, J = 5.2 Hz, 1H), 5.48 (s, 1H), 3.96 (m, 1H), 3.72 (bs, 1H), 2.44 (m, 2H), 1.95 (s, 3H), 1.81 (m, 9H), 1.07 (d, 9H), 0.94 (bs, 2H), 3.40 and 1.60 ppm (1H each exchanged with D_2O). The data for **1** are consistent with 3-angelyl-20-deoxyingenol (figure).

A minor substance, compound **2**, was also isolated from the ether fraction, R_f 0.30 and was identified as 3-hexanoyl-20-deoxyingenol from its spectral data; m.s. (chemical ionisation, CH_4, 150°): m/z 431 (15%, $M^{+\cdot}+1$), 430 (2%, $M^{+\cdot}$), 315 (100%), 314 (25%), 297 (40%) and 296 (25%); NMR ($CDCl_3$, 100 MHz): δ 6.08 (s, 1H), 5.78 (d, J = 5.1 Hz, 1H), 5.48 (s, 1H), 4.0 (m, 1H), 3.80 (bs, 1H), 2.40 (m, 2H), 2.31 (m, 2H), 1.80 (bs, 6H), 1.24 (s, 6H), 1.06 (d, 9H), 0.90 (t, 5H), 3.42 and 1.61 ppm (1H each exchanged with D_2O).

Compounds **1** and **2** were hydrolysed to the polyol **3** by means of 0.5 M methanolic KOH at room temperature. **3** was converted[11] to its diacetate **4** by reaction in acetic anhydride/pyridine (2/1) and was identified as 20-deoxy ingenol-diacetate, R_f 0.51; ei-ms: $M^{+\cdot}$ at m/z 416 (5%, $C_{24}H_{32}O_6$).

A 2nd minor component **5** of the ether fraction was identified as 3-angelyl-ingenol, R_f 0.4 (Kieselguhr G. 500-µm coated with 20% diethylene glycol, solvent heptane/benzene (9/1)); m.s. (chemical ionisation, CH_4, 150°): m/z 431 (10%, $M^{+\cdot}+1$), 430 (4%, $M^{+\cdot}$), 413 (30%), 412 (3%), 313 (100%), and 295 (60%); ei-ms: exhibited no $M^{+\cdot}$ ion but fragment ions were evident at m/z 312 (60%, $C_{20}H_{24}O_3$), 296 (40%) and 121 (100%, C_8H_9O); NMR ($CDCl_3$, 100 MHz): δ 6.16 (m, 1H), 6.06 (s, 1H), 5.72 (d, J = 5.) Hz, 1H), 5.50 (s, 1H), 4.30 (ABq, 2H, J = 4.3 Hz), 4.24 (m, 1H), 3.68 (bs, 1H), 2.44 (m, 2H), 1.92 (m, 6H), 1.79 (m, 3H), 1.26 and 1.14 (s, 3H each), 0.98 (d, 5H), 3.48, 3.16 and 1.62 ppm (1H each, exchanged with D_2O), **5** was converted to the polyol **6** by means of base catalysed hydrolysis and **6** was converted to its triacetate **7** with acetic anhydride/



Compound		R^1	R^2	R^3	ID_{50} 2 h	ID_{50} 24 h
1	3-Angelyl-20-deoxyingenol	Angelate	CH_3	OH	0.09 µg	0.18 µg
2	3-Hexanoyl-20-deoxyingenol	Hexanoate	CH_3	OH	0.19 µg	0.26 µg
3	20-Deoxyingenol	OH	CH_3	OH	–	–
4	20-Deoxyingenol-diacetate	Acetate	CH_3	Acetate	–	–
5	3-Angelylingenol	Angelate	CH_2OH	OH	1.16 µg	4,7 µg
6	Ingenol	OH	CH_2OH	OH	–	–
7	Ingenol triacetate	Acetate	$CH_2O-COCH_3$	Acetate	–	–

pyridine (2/1). Ingenol triacetate **7** was crystallised from methanol, m.p. 196 °C; m.s. (electron impact): m/z 474 ($M^{+\cdot}$, 2.5%, $C_{26}H_{34}O_8$), 456 (2%), 414 (16%), 354 (40%), 312 (93%), 294 (86%) and 121 (100%); IR (solid film KBr disc): γ_{max}, 3430, 1740, 1705 and 1640 cm^{-1}; C.D. (methanol): (210 $[\theta] = +5148$, 224 $[\theta] = -20064$, 298 nm $[\theta] = +3003$).

The acyl groups in the case of compounds **1, 2** and **5** were assigned to the C-3 position on the basis of the comparison of their NMR-spectra to those of other ingenane diterpenes[6].

The irritant dose 50% (ID_{50}) on mouse ears was determined for the 3 pure compounds (figure) both 2 h and 24 h after application to the test animals. The onset of erythema was rapid and the majority of the compounds were biologically active in sub-µg-doses. In addition the erythema persisted for 24 h and was still measurable after this time. This is in contrast to the daphnane esters isolated from some *Euphorbia* species[13] and it is possible that ingenane esters cause more extensive tissue damage. Compounds **1, 2,** and **5** were also found to be about as potent as podophyllin in inhibiting the uptake of ^{3}H-thymidine by TLX/5 mouse lymphoma cells. These compounds therefore represent the irritant and cytotoxic constituents of *E. paralias,* but they may be readily separated from the commercially required hydrocarbon fraction by solvent partition.

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Serum dopamine-beta-hydroxylase activity in a new strain of spontaneously hypertensive rats[1]

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Summary. The serum dopamine-β-hydroxylase (DBH) activity is higher in 5-week-old rats of the Lyon Hypertensive strain than in rats of the Lyon Normotensive strain. This difference disappears in older animals when the hypertension is developed, while the DBH activity decreases in the both strains.

The noradrenaline synthesizing enzyme, dopamine-β-hydroxylase (DBH), is contained in the vesicles of the noradrenergic nerves and released with noradrenaline by exocytosis[3,4]. Therefore, this enzyme is present in the blood and the determination of the circulating DBH activity has been used as an index of the sympathetic nervous system tone[4].

Thus, it was of interest to measure this parameter in order to evaluate the participation of the peripheral noradrenergic nerves in experimental hypertension. The aim of this work was to study serum DBH activity in a new strain of spontaneously hypertensive rats[5] at different ages which characterize the development of hypertension.

Male rats of the 13th generation of the Lyon Hypertensive strain (LH rats) or Lyon Normotensive strain (LN rats) were used at the ages of 5, 9 and 21 weeks. The day before sacrifice, the systolic blood pressure was measured by a tail cuff plethysmographic method (Narco Biosystem) in preheated (10 min at 36 °C) rats. The animals were sacrificed by a blow on the head; then blood was obtained by cardiac puncture, centrifuged (+4 °C) and the serum used for the determination of the DBH activity.

The DBH activity was measured according to a modification of the method of Molinoff et al.[6]. The assays were performed on 10 µl of 5-fold diluted serum; 5 µl of 25×10^{-5} M $CuSO_4$ were added, followed by 10 µl of a reaction mixture containing: 2 µl of 0.5 M sodium fumarate (pH = 5.0), 2 µl of 12 mM sodium ascorbate, 1 µl of 7.5 mM pargyline, 1 µl of catalase (260 units), 2 µl of 0.4 M sodium acetate buffer pH = 5.0 and 2 µl of 6.25 mM tyramine. Boiled serum was used for the blanks. The incubation was carried out at 37 °C for 45 min and was stopped by cooling the tubes in an ice-water bath. Then, 10 µl of a 2nd reaction mixture were added, containing 5.5 µl of 1 M Tris HCl buffer pH = 8.6, 2.5 µl of 0.1 M sodium ethylenediaminetetracetate, 1 µl of phenylethanolamine-N-methyltrans-

Systolic blood pressure, body weight and serum dopamine-β-hydroxylase activity in spontaneously hypertensive (LH) and normotensive (LN) rats at different ages

Age (weeks)	Rat strain	Systolic blood pressure (mm Hg)	Body weight (g)	DBH activity (nmole/h/ml)
5	LN	103.1 ± 2.3	87 ± 4	15.14 ± 1.07 (10)
	LH	114.8 ± 1.8**	91 ± 9	19.36 ± 1.49* (9)
9	LN	120.8 ± 3.1	216 ± 8	4.58 ± 0.44 (14)
	LH	145.6 ± 5.4***	233 ± 9	4.87 ± 0.47 (14)
21	LN	128.5 ± 4.9	320 ± 8	2.98 ± 0.26 (7)
	LH	173.5 ± 4.1***	390 ± 10***	3.29 ± 0.38 (7)

The values are expressed as mean ± SEM and the number of animals is in brackets. The DBH activity is expressed in nmoles of tyramine transformed per h and per ml of serum. The statistical differences between LN and LH rats at the same age were determined by student's t-test for unpaired data and are indicated: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.