spectroscopic identification (mp IR, NMR, CD, UV and MS). We now report a method which is capable of detecting vomifoliol quantitatively, even at low concentrations. The method involves a MeOH extraction of the plant material in a Soxhlet and the separation of the mixture so obtained on an alumina column using solvent mixtures of graded and increasing polarity. PLC is then used to further purify fractions which might contain vomifoliol as judged by TLC monitoring. Identification is made by TLC comparison with natural or synthetic [7,8] vomifoliol in three solvent systems and then finally confirmed by a GLC method. This method has so far been tested on six different plants which have previously not been screened for vomifoliol.

Leaves and stems of *Croton linearis* Jacq. (Euphorbiaceae), *Blighia sapida* Konig (Sapindaceae), *Murraya paniculata* (L) Jack (Rutaceae) and *Eichhornia crassipes* (Mart) Solms (Pontederiaceae) have been shown to contain vomifoliol. The bark of *Gliricidia sepium* (Papilionaceae) and the fruit of *Persea americana* Mill (Lauraceae) were shown to be negative.* A typical procedure is given in the experimental for material from *Blighia sapida*. In the case of the avacado pears (*Persea americana*) an additional defatting stage, using petrol (40–60°) was carried out in the Soxhlet extractor prior to the MeOH extraction.

EXPERIMENTAL

Blighia sapida, dried milled leaves and twigs (120 g) was extracted in a Soxhlet continuously with 700 ml MeOH until no further colouring material was extracted (3 days). Solvent removal yielded 9 g of crude material. This was placed on a Grade II–III Al_2O_3 column, (4 × 45 cm) in a minimum of MeOH and then eluted with pure solvents and solvent mixtures (C_6H_6–EtOAc–MeOH) of increasing polarity. Seven 1l fractions were collected as follows: *1*, C_6H_6; *2*, C_6H_6–EtOAc (9:1); *3*, C_6H_6–EtOAc (1:1); *4*, EtOAc; *5*, EtOAc–MeOH (1:1); *6*, EtOAc–MeOH (1:4) and *7*, MeOH. With the aid of TLC (Kiselgel 0·05–0·2 mm, 70–325 mesh) and using $CHCl_3$–EtOAc (1:6) as the developing solvent, fractions *5*, *6* and *7* were shown to contain material with similar R_f values to vomifoliol (R_f = 0·35) and were combined (1·8 g). This material was further purified by PLC on silica using the solvent system $CHCl_3$–EtOAc (1:6). Two additional TLC solvent systems, $CHCl_3$–EtOAc (1:1) (R_f = 0·23) and EtOAc–MeOH (5:1) (R_f = 0·64) were used to verify the presence of vomifoliol with the aid of an authentic marker. On TLC plates, appeared as a bright purple florescent spot under UV light (254 nm). Location was confirmed either by its reaction to iodine vapour (brown spot) or by spraying with a 2% aq: solution of 2,4-dinitrophenylhydrazine containing 4% conc. H_2SO_4, (orange spot). Further confirmation was by way of a GLC study of the PLC purified materials. In a typical run a 2 m × 3 mm, 3% SE-30 column was used with He at 30 ml/min; runs were isothermal at 170°, with injection port temperature of 250°, and detection (FID) temperature of 270°. Sample size varied from 0·2–2·0 μl with EtOAc as solvent and identity confirmed with synthetic vomifoliol as marker . In preliminary experiments it was shown that other naturally occurring compounds such as farnesol [9], geraniol and abscisic acid (ABA) could be efficiently separated from vomifoliol by this GLC system: (R_t) for these compounds: geraniol, 2 min; *cis-trans*-farnesol, 10 min; *trans-trans*-farnesol, 11 min; vomifoliol, 13 min; ABA > 20 min.

*All plant material was identified by the Herbarium, Botany Department, U.W.I., Jamaica.

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TWO NEW TOXINS FROM THE LATEX OF *EUPHORBIA POISONII*

FRED J. EVANS and RICHARD J. SCHMIDT

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39, Brunswick Square, London WC1N 1AX, U.K.

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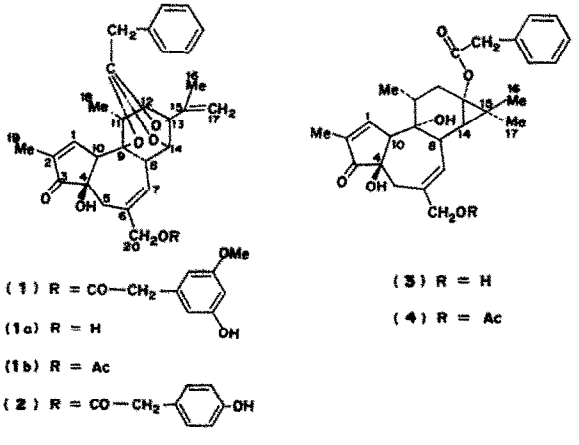
The latex of *Euphorbia poisonii*, known as "Tinya" (Hausa), has been used in West Africa as an arrow fish poison [1]. It produces lesions of the skin in both man and livestock which are susceptible to secondary infections. It also causes inflammation of the mucus membranes of the nose, mouth and eyes and in chronic exposure leads to blindness. A biological screen of Nigerian succulent species [1] suggested that on mouse skin the inflammation produced, although acute initially, did not persist for up to 24 h as is normally the case for diterpene

stimulated hyperplasia. A series of toxins were isolated from the latex and we report here the structure of four of these, two of which are new natural products.

Plant material. E. poisonii latex was collected from the Vom district of the Jos plateau in Nigeria.

Present work. A clear glassy resin was isolated from 500 ml of latex as previously described [2,3], using combinations of partition and adsorption chromatographic techniques. The resin was separated into several components, four of which were major products. These toxins were pure by TLC [3,4] and homogenous by means of MS, although they failed to crystallise from several solvents. Each of these compounds produced severe inflammation of mouse skin when tested by traditional techniques [5].

Compound (1) yield 120 mg, produced an orange–black spot (R_f 0·43) by TLC (20% EtOAc in cyclohexane Kieselguhr G. 0·75 mm 120 for 1 hr impregnated with 20% Digol in Me_2CO) when sprayed with 60% H_2SO_4. High resolution electron impact MS produced an M^+ ion in the spectrum at *m/e* 628 (relative abundance 27%, $C_{37}H_{40}O_9$) and significant fragment ions in the upper region of the spectrum at *m/e* 610 (R.A. < 5%, $M^{+\cdot}$-[18]), 492 (R.A. 5%, $C_{29}H_{32}O_7$, $M^{+\cdot}$-[136]); 474 (R.A. 5%, $M^{+\cdot}$-[136 + 18]); 446 (R.A. 6%, $C_{28}H_{30}O_5$, $M^{+\cdot}$-[182], 428 (R.A. 5%, $M^{+\cdot}$-[182 + 18]); 310 (R.A. 36%, $C_{20}H_{22}O_3$, $M^{+\cdot}$-[182 + 136]); 292 (R.A. < 5%, $M^{+\cdot}$-[182 + 136 + 18]). (Base peak *m/e* 137). The MW was confirmed by field desorption MS there the base peak was produced at *m/e* 628 together with an M + 1 ion at *m/e* 629 (30% R.A.). The UV spectrum had λ_{max}^{MeOH} at 238 nm (log 3·9) and 290 nm (log 3·5), (bathochromic shift with addition of NaOH to 305 nm); in the IR spectrum (KBr microcells) ν_{max} were exhibited at 3460, 2950, 1740, 1715, 1520, 1275, 1240, 1150, 1030, 910, 740 and 705 cm^{-1}. In the NMR spectrum (60 MHz, $CDCl_3$, δ, TMS = 0·00 ppm) signals were produced at δ 0·96*d* (*J* = 10 Hz, 3H-18; δ 1·53*s* (3H-16); δ 1·82*m* (3H-19); δ 2·08–2·3*m* (2H-12); δ 2·32–2·15 AB (2H-5); δ 2·69*m* (H-11); δ 3·06*m* (H-8, H-10); δ 3·22 (2H, ϕ–CH_2–); δ 3·54 (2H, ϕ–CH_2–); δ 3·86*s* (3H, ϕ–OMe); δ 4·20*d* ($J_{14/8}$ = 6·5 Hz, H-14); δ 4·56 AB (2H-20); δ 4·72 AB (2H-17); δ 5·87*m* (H-7); δ 6·8*s* (3-aromatic protons); δ 7·28*m* (5-aromatic protons); δ 7·44*m* (H-1); δ 5·60 and δ 2·33, 2-OH deuterium exchange. When irradiated at δ 3·19 the doublet at δ 4·20 changed to a singlet at δ 4·36.



Diterpenes of *E. poisonii*.

Compound (1) on hydrolysis produced (1a). In its MS (1a) exhibited an M^+ ion at *m/e* 464 (R.A. 10%) and significant fragment ions at *m/e* 446 (9%, $M^{+\cdot}$-[18]); 428 (5%, $M^{+\cdot}$-[36]); 328 (45%, $M^{+\cdot}$-[136]); 310 (100%, $M^{+\cdot}$-[136 + 18]); and *m/e* 292 (18%, $M^{+\cdot}$-[136 + 36]). The NMR was similar to (1) with the exception that the 3 aromatic H signals at δ 6·8, the 2H singlet at δ 3·54 and the δ 3H singlet at δ 3·86 were absent. An extra hydroxyl H was observed at δ 1·83 and the allylic 2H singlet at δ 4·56 in (1) was evident at δ 4·10. In the IR spectrum (KBr discs) ν_{max} were exhibited at 3450, 2940, 1705, 1025, 1005, 910, 740 and 700 cm^{-1}. Acetylation of (1a) produced the mono-acetate (1b), which in its MS exhibited at M^+ ion at *m/e* 506 (18%) and significant fragment ions at *m/e* 448 (<5%, $M^{+\cdot}$-[18]); 446 (17%, $M^{+\cdot}$-[60]); 428 (7%, $M^{+\cdot}$-[60 + 18]); 370 (100%, $M^{+\cdot}$-[136]); 352 (11%, $M^{+\cdot}$-[136 + 18]); 310 (71%, $M^{+\cdot}$-[136 + 60]); 292 (43%, $M^{+\cdot}$-[60 + 136 + 18]. The NMR spectrum of (1b) exhibited an additional 3H signal due to acetate at about δ 2·1. After hydrolysis the substituted phenyl acetic acid moiety was identified as its methyl ester by means of GC-MS. Compound (1) was assigned as the orthoester resiniferatoxin (1) [6].

Compound (2), yield 6 mg, also produced an orange–black staining spot by TLC (R_f 0·3) when sprayed with 60% H_2SO_4. High resolution electron impact MS produced an M^+ ion at *m/e* 598 ($C_{36}H_{38}O_8$) and significant fragment ions at *m/e* 580 (<5%, $M^{+\cdot}$-[18]); 462 (8%, $M^{+\cdot}$-[136]); 444 (<5%, $M^{+\cdot}$-[136 + 18]); 446 (10%, $M^{+\cdot}$-[152]); 428 (<5%, $M^{+\cdot}$-[152 + 18]; 310 (90% $M^{+\cdot}$-[152 + 136]); 292 (13%, $M^{+\cdot}$-[152 + 136 + 18]), (relative to base peak *m/e* 107). The MW was confirmed by field desorption MS where the 100% ion was observed at *m/e* 598,. The IR, UV spectra of (2) were similar to (1). In the NMR spectrum (2) (100 MHz, $CDCl_3$, δ, TMS = 0·00 ppm)., exhibited signals at δ 0·96*d* (*J* 9Hz, 3H-18); δ 1·52 (3H-16); δ 1·81*m* (3H-19); δ 2·08–2·3*m* (2H-12); δ 2·10, 2·30 AB (2H-5); δ 2·56*m* (H-11); δ 3·06*m* (H-8; H-10); δ 3·20 (2H, ϕ–CH_2–); δ 3·54 (2H, ϕ–CH_2–); δ 4·18*d* (*J* 5·5 Hz, H-14); δ 4·56 AB (2H-20); δ 4·72 AB (2H-17); δ 5·88*m* (H-7); δ 6·2–7·4 (9 aromatic H); δ 7·44*m* (H-1), δ 5·30, δ 2·32 2-OH deuterium exchange. Upon hydrolysis in MeOH (2) was converted to (1a) (TLC, MS, NMR, IR, UV). The substituted phenylacetate at C-20 was identified as its methyl ester by GC–MS after hydrolysis of (2). Compound (2) was assigned as a new orthoester diterpene which we propose to call tinyatoxin (2).

Compound (3), yield 145 mg, produced an orange staining spot by TLC (R_f 0·15) when sprayed with 60% H_2SO_4. In the MS of (3) the M^+ ion was exhibited at *m/e* 466 ($C_{28}H_{36}O_6$, 7%), with fragment ions in the upper region of the spectrum at *m/e* 448 (27%, $M^{+\cdot}$-[18]; 430 (7%, $M^{+\cdot}$-[36]); 412 (20%, $M^{+\cdot}$-[54]); 330 (40%, $M^{+\cdot}$-[136]); 312 (100%, $M^{+\cdot}$-[136 + 18]); 294 (67%, $M^{+\cdot}$-[136 + 36]). MW was confirmed by field desorption MS where the 100% ion was produced at *m/e* 466. The IR, UV and CD spectra were similar to several other esters of 12-deoxy-4βOH-phorbol [2]. In the NMR spectrum (3) produced signals at δ 7·6*m* (H-1); δ 7·31*s* (5 aromatic protons); δ 5·62*d* (*J* 6Hz, H-7); δ 3·97 AB (2H-20); δ 3·61 (2H, ϕ–CH_2–); δ 3·22*m* (H-10); δ 3·0*m* (H-8); δ 2·50 *br* (2H-5); δ 1·9–2·18*m* (2H-12); δ 1·76*m* (3H-19); δ 1·03 *br* (6H, 16 and 17); 0·86*d* (*J* 9Hz, 4H-18, 14), δ 5·31, δ 2·67 and δ 2·0 (three OH, deuterium exchange). Compound (3) was resistant to mild hydroly-

sis but on hydrolysis with saturated $Ba(OH)_2$ in MeOH, an alcohol was produced which after acetylation was identified as 12-deoxy-phorbol-13, 20-diacetate (TLC, GC, MS, CD, IR, NMR) [2], and also an acid identified as phenylacetic acid by GC-MS of its methyl ester. On acetylation (3) produced the mono-acetate (4), and was assigned as the new natural product 12-deoxy-4βOH-phorbol-13-phenyl acetate.

Compound (4), yield 460 mg, was a high migrating, orange staining spot by TLC (R_f 0·53). In the MS of (4) an M^+ ion was produced at *m/e* 508 (12%, $C_{30}H_{36}O_7$) and significant fragment ions at *m/e* 490 (6%, $M^{+\cdot}$-[18]); 448 (10%, $M^{+\cdot}$-[60]); 430 (5%, $M^{+\cdot}$-[60 + 18]); 372 (28%, $M^{+\cdot}$-[136]); 354 (14%, $M^{+\cdot}$-[136 + 18]); 312 (100%, $M^{+\cdot}$-[136 + 60]); 294 (95%, $M^{+\cdot}$-[136 + 60 + 18]). In the NMR spectrum (4) exhibited signals similar to (3) with the addition of a 3H signal due to an acetate methyl at δ 2·02, and also a shift of the allylic 2H signal at δ 3·97 in (3) to δ 4·47 in (4). Hydrolysis of (4) produced (3) (TLC, MS, CD, NMR), and 12-deoxy-4βOH-phorbol was identified as its diacetate after total hydrolysis as before. Compound (4) was identified as 12-deoxy-4βOH-phorbol-13-phenylacetate-20-acetate (4) [7].

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TERPENOIDS FROM *ELAEAGNUS OLDAHMI*

TSUEN-IH RUO and HIROSHI KAKISAWA
Department of Chemistry, Tokyo Kyoiku University, Otsuka, Tokyo
and
YU PAN CHEN and HON YEN HSÜ
Brian Research Institute of Taiwan, 116 Chun-Ching South Road, Taipei, Taiwan

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Plant. Elaeagnus oldahmi Maxim was collected in Chia-ih, Taiwan on June 1970. A voucher specimen is deposited in the Herbarium of Brian Institute of Taiwan, Taipei. *Uses.* The roots of the plant is used to cure rheumatism in Taiwan. *Previous work.* None.

Present work. Powdered air-dried roots of *Elaeagnus oldahmi* were successively extracted with *n*-hexane and $CHCl_3$. The hexane extract afforded sitosterol. The concentrated $CHCl_3$ extract was separated to neutral and acidic parts. The neutral part contained sitosteryl glucopyranoside. The acidic part was methylated with CH_2N_2, and the esters fractionated by column chromatography on silicic acid to give two triterpenoid esters. The first ester was identified as methyl arjunolate [1] from the following properties: $C_{31}H_{50}O_5$; mp 208–210°; $[\alpha]_D$ +58·20; ν 3450, 1730 cm^{-1}; δ 0·73 (*s*, 3H), 0·81 (*s*, 3H), 0·94 (*s*, 3H), 0·95 (*s*, 3H), 1·08 (*s*, 3H), 1·15 (*s*, 3H), 3·62 (*s*, 3H), 5·3 (*brd*, 1H); MS 502(M^+), 262, 222, 203 (base peak): triacetate; ν 1750 cm^{-1}, δ 0·74 (*s*, 3H), 0·91 (*s*, 6H), 0·94 (*s*, 3H), 1·10 (*s*, 3H), 1·13 (*s*, 3H), 2·00 (*s*, 3H), 2·03 (*s*, 3H), 2·10 (*s*, 3H), 3·63 (*s*, 3H), 3·73 (AB *q*, *J* 12 Hz, 5·2 (*m*, 1H).

The second ester was shown to be methyl maslinate [2] (methyl crategolate) from the following properties: $C_{31}H_{50}O_4$, mp 215–219°; ν 3350, 1730 cm^{-1}; δ 0·72 (*s*, 3H), 0·81 (*s*, 3H), 0·90 (*s*, 3H), 0·92 (*s*, 3H), 0·96 (*s*, 3H), 1·01 (*s*, 3H), 1·12 (*s*, 3H), 3·55 (*s*, 3H), 5·18 (*br.s*, 1H), MS 486 (M^+), 262, 224, 203: diacetate: ν 1742, 1725 cm^{-1}, δ 0·72 (*s*, 3H), 0·92 (*s*, 12H), 1·07 (*s*, 3H), 1·12 (*s*, 3H), 1·98 (*s*, 3H), 2·05 (*s*, 3H), 3·55 (*s*, 3H), 4·7 (*d*, 1H, *J* 11 Hz), 5·20 (*br.s*, 1H). The IR spectra of the second ester and its acetate were coincident with spectra of authentic methyl maslinate and its diacetate, respectively.

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