of which corresponds to plenolin (**9**). The preceding evidence leads definitively to **5** and **6**, respectively, for the structures and stereochemistries of microhelenin-B and -C.

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# A NEW PHORBOL TRIESTER FROM THE LATICES OF *EUPHORBIA FRANKIANA* AND *E. COERULESCENS*

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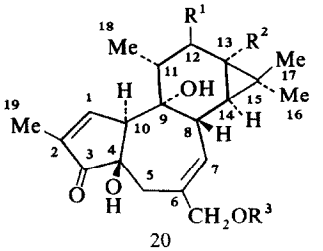
**Key Word Index**—*Euphorbia frankiana*; *E. coerulescens*; Euphorbiaceae; diterpene; 12-*O*-iso-butyl-phorbol-13-acetate 20-angelate.

*Euphorbia frankiana* Berg. and *E. coerulescens* Haw. are both succulent African species of the subdivision *Polygonae* (subsection *Diacanthium*) of the genus *Euphorbia* [1]. A phytochemical investigation of species of this subdivision [2] indicated that the triterpene profile was uniform; however the diterpene profile was more complex [3]. The subdivision contains species which produce both tigliane, ingenane and daphnane diterpenes [3] but only the species *E. frankiana* and *E. coerulescens* have yielded phorbol to date [3], [4]. The latices of these plants are irritant to skin [5] and the major toxins which are esters of 12-deoxyphorbol have been described [6]. This communication describes the identification of a new cryptic irritant based upon phorbol, which was isolated from the methanol preserved fresh latex.

Water and methanol were removed from the preserved latices by reduced pressure distillation below 40°. The residue was exhaustively extracted with acetone, the acetone removed as before to yield a cream solid residue (40% w/w of dried latex). The crude acetone extract of *E. frankiana* had an irritant dose 50% ($ID_{50}$) [7] on mice ears of 3 μg/5 μl/ear and of *E. coerulescens* of 7 μg/5 μl/ear. The solid residues from acetone were partitioned between MeOH–$H_2O$ and hexane followed by MeOH–$H_2O$ and ether as previously described [8]. Residues from the ether phase were separated by column chromatography [8] on florosil into two fractions. The less polar fraction consisted of diesters of 12-deoxy-phorbol [9] together with smaller amounts of phorbol ester (**1**), and the polar fraction consisted of 12-deoxy-phorbol monoesters [8].

*12-O-isobutyl-phorbol-13-acetate-20-angelate* (**1**). *E. frankiana* contained 0.14% w/w of acetone-soluble residue: *E. coerulescens* contained 0.01% w/w of acetone-soluble residue. The non-polar fraction from column chromatography was subjected to PLC on silica gel G (500 μm layers) buffered at pH 7.0, using first $CHCl_3$-ether-$C_6H_6$ (1:3:3) (H $R_f$ 60) and then after recovery $CHCl_3$–acetone–$C_6H_6$ (95:6:50) (h $R_f$20). Compound (**1**) which was still contaminated with esters of 12-deoxy-phorbol was further purified by partition chromatography using digol as stationary phase [10] and finally by repeated elution PLC on silica gel as before using $C_6H_6$–$C_6H_{12}$–ether–EtOAc (4:8:3:6). After elution a resin was obtained which produced one spot by analytical TLC (h $R_f$ 67) in the above system. This substance exhibited a yellow colour in UV light after spraying with 60% aqueous $H_2SO_4$, and a pink colour in daylight after spraying with MeOH–$H_2SO_4$ (1:1) and heating. In the MS (**1**) exhibited an $M^{+\cdot}$ at *m/e* 558 ($C_{31}H_{42}O_9$) and fragment ions at *me* 498 (10%); 471 (14%); 470 (8%); 458 (10%); 452 (2%); 410 (35%); 398 (16%); 370 (40%); 310 (100%) and 292 (38%). Below the ion at *m/e* 292 the spectrum was similar to that of phorbol triacetate [3].

The IR spectrum (KBr discs) exhibited $\nu_{max}$ at 3405; 3000; 1730 (*br*); 1625 $cm^{-1}$. In the NMR spectrum (60 MHz. $CDCl_3$. TMS internal standard) signals were evident at $\delta$ 7.61 *s*, broad 1H; $\delta$ 6.08 *m*, 1H; $\delta$ 5.71 *d* ($J = 5$ cps) 1H; $\delta$ 5.30 *d* ($J = 2$ cps) 1H; $\delta$ 4.47 *s*, 2H; $\delta$ 3.1–3.3 *m*, 2H; $\delta$ 2.57 *s*, *br*, 1H; $\delta$ 2.08 *s*, 3H; $\delta$ 1.95 *m*, 6H; $\delta$ 1.80 *m*, 3H; $\delta$ 1.23 *s*, 6H; $\delta$ 1.16 *s*, 6H; $\delta$ 0.97 *d* ($J = 3$ Hz), 4H; $\delta$ 5.52, OH (deuterium exchange) ppm. Hydrolysis of (**1**) with saturated $Ba(OH)_2$ in MeOH overnight yielded the parent alcohol, identified as phorbol by its triacetate. (TLC, GLC, CD, MS, NMR), and the acyl




20

| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **1** | Isobutyrate | Acetate | Angelate |
| **2** | " | " | H |

moieties isobutyric and angelic acids identified as their methyl esters (GLC–MS). These data suggested that ester (**1**) was a triester of the tigliane diterpene phorbol, and further that the acyl groups consisted of isobutyric, angelic and acetic acids. In view of the ion in the MS of (**1**) at *m/e* 471 it seemed most likely that isobutyric acid had fragmented as an acyloxy radical and this acid was therefore located at C-12 of phorbol [11]. This was further confirmed by the IR spectrum which exhibited a broad single maxima at around 1730 $cm^{-1}$ similar to Hecker's compound A′ (12-O-tetradecanoylphorbol-13-acetate) [11]. Acid catalysed transesterification of (**1**) [12] produced (**2**) a low $R_f$ value ester (h $R_f$ 4 in system above). Comparison of TLC migration rates have shown [13] that removal of the primary ester function at C-20 produced highly polar derivatives with low h $R_f$ values. In the MS of (**2**) an $M^{+\cdot}$ ion was exhibited at *m/e* 476 and fragment ions corresponding to the loss of acetate (*m/e* 416) and isobutyrate (*m/e* 389 and *m/e* 388). The NMR spectrum of (**2**) was similar to (**1**) with the exceptions that signals due to angelic acid were absent and that the 2H *s* at $\delta$ 4.47 due to the primary ester protons of (**1**) were exhibited upfield at $\delta$ 4.05, thereby confirming that angelic acid was located at C-20 in ester (**1**).

Compound (**1**) was therefore assigned as the new natural product 12-*O*-isobutyl-phorbol-13-acetate-20-angelate (**1**). This triester belongs to the group of toxins known as cryptic irritants [12], which are characterised by weak inflammatory effects on skin unless the C-20 acyl group is removed by hydrolysis.

*Plant Material*

*E. frankiana* and *E. coerulescens* latex collected into methanol from plants growing at The Royal Botanical Gardens, Kew, England.

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