CHARACTERIZATION OF AN IRRITANT 4-DEOXYPHORBOL DIESTER FROM *EUPHORBIA TIRUCALLI*[1]

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ABSTRACT.—Latex of *Euphorbia tirucalli*, collected in Colombia, yielded 12-*O*-2*Z*-4*E*-octadienoyl-4-deoxyphorbol-13-acetate (**1**), which exhibited an irritant potency equivalent to that of the standard irritant, phorbol-12-tetradecanoate-13-acetate, in a mouse ear test system. The unsaturated 4-deoxyphorbol diesters recently reported in *E. tirucalli* grown in South Africa were not observed in the present study.

Euphorbia tirucalli L. (Euphorbiaceae) is a succulent household plant, commonly known as pencil cactus, which exudes a caustic latex (1). This latex has been shown to produce uveitis and keratoconjunctivitis in the eyes of dogs (2) and also to exhibit a potent tumor-promoting activity on mouse skin (3). *E. tirucalli* has been used as a cure for warts in India and Brazil (4), and a proposal has been made to grow this species in desert regions of the United States as a source of fuel (5).

In the present work an acetone extract of *Euphorbia tirucalli* was shown to exhibit a potent irritant effect on mouse ears. The irritant activity was concentrated in ether, and a novel compound, 12-*O*-2*Z*-4*E*-octadienoyl-4-deoxyphorbol-13-acetate (**1**), was obtained by column chromatography followed by preparative thin-layer chromatography. The relative positions of the ester functions and the identity of the diterpene moiety were established by hydrolysis and acetylation. Recently, the long-chain ester substituent in **1** has been found in *E. tirucalli* as part of an ester of a different diterpene parent alcohol (6).

EXPERIMENTAL[2]

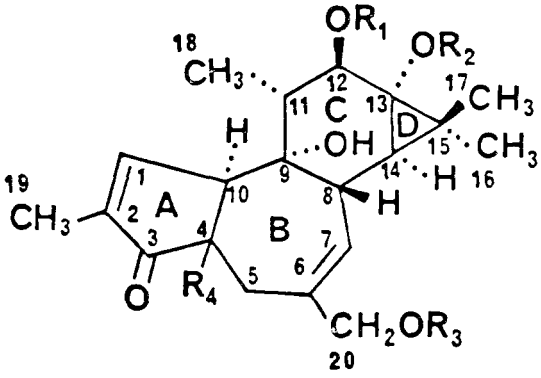
PLANT MATERIAL.—Latex of *Euphorbia tirucalli* L. was collected near Medellin, Colombia, in November 1977 and stored during transit in methanol. The plant was identified by Prof. G. L. Webster, Department of Botany, University of California, Davis, CA. Voucher specimens of the aerial parts of the plant are deposited in the Herbarium of the Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, Chicago, IL.

EXTRACTIONS AND FRACTIONATION.—Dried *Euphorbia tirucalli* latex (17.4 g) was extracted six times with acetone at room temperature; the acetone extract had an irritant dose 50% (ID_{50}) of 1.7 μg/5 μl after 24 hours in a mouse ear test system (7). The residue was dissolved in 100 ml methanol-water (9:1) and partitioned with 2×50 ml petroleum ether (b.p. 60°). No irritant activity was detected in the non-polar fraction. Water (80 ml) was added to the lower layer, which was then extracted with 3×100 ml ether. The ether layer, which contained all the irritant activity (ID_{50} 0.12 μg/5 μl after 24 hr), was fractionated by gradient elution chromatography on Florisil[3] (100–200 mesh) with mixtures of benzene and ethyl acetate. Irritant fractions were subjected to partition tlc on kieselguhr G[4], with diethylene glycol as stationary phase (8), in cyclohexane-methyl ethyl ketone (17:3). A zone (R_F 0.70) was ex-

[1]A summary of the present work was presented at the poster sessions of the First Joint Meeting of the American Society of Pharmacognosy and the Phytochemical Society of North America held at Stillwater, Oklahoma, August 14–17, 1978.

[2]Uv spectra were obtained with a Beckman model DB-G grating spectrophotometer. The ir spectra were determined using a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. PMR spectra were recorded in $CDCl_3$ with a Varian model T-60A instrument, operating at 60 MHz, with a Nicolet model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard, and chemical shifts are reported in δ (ppm) units. Low resolution mass spectra were obtained on a Hitachi Perkin-Elmer, model RMU-6D single-focusing instrument, operating at 70 eV.

[3]Fisher Scientific Co., Itasca, Illinois.



tracted into acetone, separated from diethylene glycol (8), and purified by preparative tlc on buffered silica gel G[4] plates (pH 7.0) in methylene chloride-hexane-acetone (3:1:1). Elution of a band with R_F 0.52 with acetone afforded 12-*O*-2*Z*-4*E*-octadienoyl-4-deoxyphorbol-13-acetate (**1**, 15 mg).

CHARACTERIZATION OF **1**.—The resinous 12-*O*-2*Z*-4*E*-octadienoyl-4-deoxyphorbol-13-acetate (**1**) exhibited the following spectral properties: ir, ν max ($CHCl_3$) 3660, 3560, 3420, 1715, 1635, 1000, 965 cm^{-1}; uv max (MeOH) 262 nm (log ϵ 4.49); pmr ($CDCl_3$) δ 0.94 (*H*, d, *J*=6 Hz, 14-*H*), 0.98 (3*H*, d, *J*=6 Hz, 18-CH_3), 1.21, 1.26 (6*H*, s, 16-CH_3, 17-CH_3), 1.73 (3*H*, m, 19-CH_3) 2.12 (3*H*, s, $-COCH_3$), 2.17 (2*H*, m, 6'-CH_2), 3.24 (*H*, m, 10-*H*), 3.71 (*H*, br. s., exchanged with D_2O, *OH*), 4.01 (2*H*, s, 20 H_2), 5.48 (*H*, d, *J*=9.5 Hz, 12-*H*) 5.53 (*H*, d, *J*=9.5 Hz, 2'-*H*), 5.60 (*H*, m, 7-*H*), 6.12 (*H*, m, 5'-*H*), 6.59 (*H*, dd, *J*=11, 11 Hz, 3'-*H*) 7.28 (*H*, dd, *J*=11, 16 Hz, 4'-*H*), and 7.57 (*H*, m. 1-*H*); ms, *m/e* M+ 512 (4%), 494 (2), 452 (9), 434 (3), 372 (7), 354 (2), 330 (4), 312 (19), 294 (18), 123 (100), 81 (24) and 43 (30). Mass measurement: found, 512.2771, calc. for $C_{30}H_{40}O_7$, 512.2773; found 312.1722, calc. for $C_{20}H_{24}O_3$, 312.1725; found 294.1623, calc. for $C_{20}H_{22}O_2$, 294.1619.

	R_1	R_2	R_3	R_4
1	$-COCH\overset{Z}{=}CH-CH\overset{E}{=}CHCH_2CH_2CH_3$ (1' 2' 3' 4' 5' 6' 7' 8')	$-COCH_3$	-H	β-H
2	$-COCH\overset{Z}{=}CH-CH\overset{E}{=}CHCH_2CH_2CH_3$ (1' 2' 3' 4' 5' 6' 7' 8')	-OH	-H	α-H
3	$-COCH_3$	$-COCH_3$	$-COCH_3$	α-H

SELECTIVE HYDROLYSIS OF **1**.—12-*O*-2*Z*-4*E*-Octadienoyl-4-deoxyphorbol-13-acetate (12 mg) was hydrolyzed in 0.02 M KOH in MeOH (0.5 ml) for 15 min at room temperature. After solvent removal under nitrogen the resinous hydrolysis product, 4-deoxy-4α-phorbol-12-2*Z*-4*E*-octadienoate (**2**, 9 mg), was purified by tlc on silica gel G[5] in methylene chloride-hexane-acetone (3:1:1) (R_F 0.15) and exhibited the following spectral data: ir, ν max ($CHCl_3$), 3580, 3380–3490, 1695, 1640, 1600, 1000, 965 cm^{-1}; uv, λ max (MeOH) 262 nm (log ϵ 4.37); pmr ($CDCl_3$) δ 1.07 (3*H*, d, *J*=8 Hz, 18-CH_3), 1.17, 1.26 (6*H*, s, 16-CH_3, 17-CH_3), 1.76 (3*H*, m, 19-CH_3), 2.04 (*H*, br. s., exhanged with D_2O, *OH*), 2.16 (2*H*, m, 6'-*H*$_2$), 2.67 (*H*, m, 4-*H*), 3.10 (*H*, m, 5a-*H*), 3.49 (*H*, m, 10-*H*), 3.68 (*H*, br. s., exchanged with D_2O, *OH*), 3.94 (2*H*, s, 20-H_2), 4.98 (*H*, d, *J*=11 Hz, 12-*H*), 5.18 (*H*, m, 7-*H*), 5.59 (*H*, d, *J*=11 Hz, 2'-*H*), 6.13 (*H*, m, 5'-*H*), 6.64 (*H*, dd, *J*=11, 11 Hz, 3'-*H*), 7.04 (*H*, br. s., 1-*H*) and 7.37 (*H*, dd, *J*=11, 16 Hz, 4'-*H*), ms, *m/e* M+ 470 (1%), 452 (1), 434 (10), 330 (27), 312 (49), 294 (12), 123 (76), 97 (97), 55 (82) and 43 (100).

HYDROLYSIS AND ACETYLATION OF **2**.—4-Deoxy-4α-phorbol-12-2*Z*-4*E*-octadienoate (**2**, 6 mg) was hydrolyzed in 0.5 M KOH in MeOH (0.5 ml) for 30 min at room temperature. Water (2 ml) and methanol (2.5 ml) were added, and 4-deoxy-4α-phorbol was extracted into 10 ml methylene chloride. On removal of solvent, the residue was acetylated at 100° with 400 μl of pyridine and 100 μl of acetic anhydride. Excess reagents were removed under nitrogen, and 4-deoxy-4α-phorbol-12,13,20-triacetate (**3**, 3 mg) was purified on silica gel[4] in benzene-ethyl acetate (2:1) (R_F 0.45) and exhibited the following spectral characteristics: pmr ($CDCl_3$) δ 0.83

[4]E. Merck, Darmstadt, W. Germany.

(*H*, d, J=6 Hz, 14-*H*), 1.05 (3*H*, d, J=6 Hz, 18-CH_3), 1.27 (6*H*, s, 16, 17-CH_3), 1.75 (3*H*, s, 19-CH_3), 2.01 (3*H*, s, -$COCH_3$), 2.06 (3*H*, s, -$COCH_3$), 2.11 (3*H*, s, ($COCH_3$), 2.47 (*H*, d, J=16 Hz, 5b-*H*), 3.26 (*H*, m, 5a-*H*), 3.46 (*H*, m, 10-*H*), 4.40 (2*H*, br. s., 20-H_2), 5.07 (1*H*, br. s., exchanged with D_2O, 9-O*H*), 5.13 (*H*, m, 7-*H*), 5.46 (*H*, d, J=10 Hz, 12-*H*) and 6.48 (*H*, m, 1-*H*); ms, *m/e* M+ 474 (0.3%), 452 (0.1), 414 (2.5), 372 (2.5), 294 (7), 284 (6), 83 (6), 57 (88), and 43 (100).

BIOLOGICAL TESTING.—Irritant dose 50% (ID_{50}) determinations of acetone solutions of fractions of *Euphorbia tirucalli* and pure diterpene esters were performed by the method of Hecker *et al.* (7) except that readings were made at both 4 hr and 24 hr after the samples were applied. Assays were carried out using male Swiss Webster mice, maintained at 20° and fed a diet of Lab-Blox (Allied Mills Inc., Chicago, IL) and water *ad libitum*. Reference diterpene irritants, phorbol-12-tetradecanoate-13-acetate and phorbol-12,13-dibenzoate, were obtained from Sigma Chemical Co., St. Louis, MO, and their ID_{50}'s and standard deviations are shown with those of **1** and **2** in table 1.

TABLE 1. *Irritant potencies of diterpene esters (determined by use of a mouse ear test system).*

Compound	Irritant Dose 50% (μg/ 5 μl)			
	4 hr	s[a]	24 hr	s[a]
1	0.04	1.24	0.07	1.28
2	>100	—	>100	—
Phorbol-12,13-dibenzoate	0.60	1.29	0.83	1.20
Phorbol-12-tetradecanoate-13-acetate	0.04	1.25	0.05	1.26

[a]s=Standard Deviation.

DISCUSSION

The mass spectrum of **1** indicated the presence of ester functions with molecular weights of 60 and 140 (8). These data, coupled with pmr observations, suggested the occurrence of acetate and octadienoate groups, respectively. The latter was assigned specifically as a 2*Z*-4*E*-octadienoyl substituent due to the superimposition of pmr data for the long-chain acid moiety of **1** with those of a previously reported phorbol ester containing the same acid (6). The stereochemical assignment of this acid by the Hecker group was based on double resonance pmr experiments and confirmation of the bond between C-2′ and C-3′ as *cis* by comparison of the downfield coupling constant with that of 2*Z*-octadecenoic acid (6, 9). Additional evidence for this moiety was obtained by comparing the ir spectrum of **1** and the four geometrical isomers of methyl decadienoate (10). The prominent absorption maxima at 965 and 1000 cm^{-1} are present only in methyl 2*Z*-4*E*-decadienoate (10).

The relative position of the two ester substituents in 12-O-2*Z*-4*E*-octadienoyl-4-deoxyphorbol-13-acetate (**1**)[5] was established by selective hydrolysis in 0.02 M KOH to yield 4-deoxy-4α-phorbol-12-2*Z*-4*E*-octadienoate (**2**). Previous work has shown that the C-12 ester substituent of phorbol diesters is less susceptible to hydrolysis than the C-13 ester substituent (12).

The parent diterpene alcohol of **1** was identified as 4-deoxyphorbol by the generation of the known compound, 4-deoxy-4α-phorbol-12,13,20-triacetate (**3**), following hydrolysis of **2** in 0.5 M KOH and subsequent acetylation. The tlc migration data of **3** determined by several developing systems (13) and the ms and pmr characteristics compared favorably with previous data (13, 14). Esters

[5]The structure of **1** and 15 other 4-deoxyphorbol esters from *Euphorbia tirucalli* were represented without stereochemical assignments or analytical back-up data (11). The complete structures of only four of these compounds have been established subsequently (15).

of 4-deoxyphorbol are known to be highly labile under basic conditions (12, 15). Evidence for the simultaneous epimerization and hydrolysis of **1** by treatment with 0.02 M KOH to form **2** was the upfield shift of the C-1 proton from δ 7.57 to 7.04 ppm and of the C-12 proton from δ 5.48 to 4.98 ppm, respectively (15).

Four 4-deoxyphorbol diesters isolated from *Euphorbia tirucalli* were recently reported with complete stereochemical assignments (15). While all of these compounds are less polar than **1**, no high R_F compounds with similar color reactions to acid sprays (13) were detected in extracts of the Colombian sample used for the present study. This observation, in combination with differences in the diterpene profile of *E. tirucalli* latices collected in South Africa and the Malagasy Republic (6, 15), raises the possibility of the existence of chemical races within this species.

Irritancy data for pure 4-deoxyphorbol esters have not been published previously. 12-*O*-2*Z*-4*E*-Octadienoyl-4-deoxyphorbol-13-acetate (**1**) was almost as potent as phorbol-12-tetradecanoate-13-acetate and more than 10 times as irritant as phorbol-12,13 dibenzoate (Table 1). The fact that **2** did not possess irritant effects emphasizes the need for a *trans*-junction between rings A and B in irritant compounds of the phorbol ester type.

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