FOUR NEW 12-DEOXYPHORBOL DIESTERS FROM *CROTON CALIFORNICUS*

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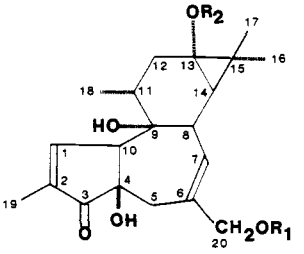
ABSTRACT.—The petroleum ether and ethanol extracts of *Croton californicus* (Euphorbiaceae) yielded, upon further fractionation, a tlc single spot complex mixture. The resolution of this mixture by reverse phase hplc gave four new 12-deoxyphorbol-13,20-diesters (**1–4**) whose identities were established by spectral properties and by chemical transformations.

The isolation and identification of two diterpenes, (−)-hardwickiic acid and (−)-methyl barbascoate, from *Croton californicus* (Euphorbiaceae) have been previously reported (1,2). In this paper the structure determinations of four new 12-deoxyphorbol diesters (**1-4**), isolated from the same source, are reported. Phorbol esters are known to occur in *Croton* species (3,4,5), while 12-deoxyphorbol esters have been reported to occur in *Euphorbia* species (6,7,8,9).

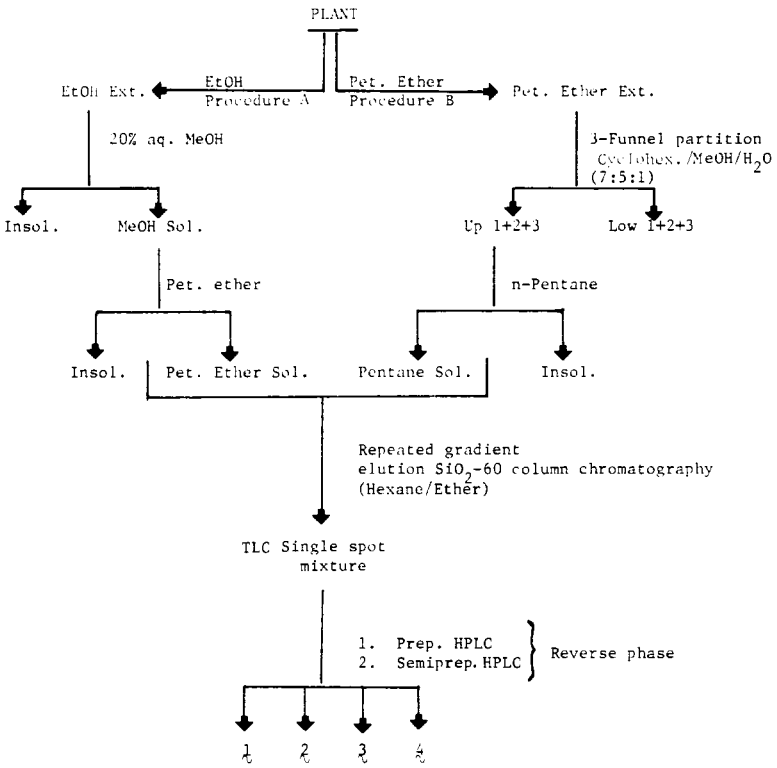
The four diesters of 12-deoxyphorbol (**1-4**) were isolated from a complex mixture (derived from the petroleum ether and ethanol extracts of the plant, as shown in scheme I) that appeared as a single spot on a normal phase tlc. The resolution of this mixture and subsequent isolation and purification of four compounds (**1-4**) were accomplished by reverse phase preparative hplc followed by semipreparative hplc. The identity of these compounds was established by spectroscopy and by chemical transformations.



	R_1	R_2
1	$CO(CH_2)_8Me$	$CO(CH_2)_8Me$
2	$CO(CH_2)_{10}Me$	
3	$CO(CH_2)_{12}Me$	
4	$CO(CH_2)_{14}Me$	
5	H	
6	Ac	Ac
7	H	H

The ir (hydroxyl band) and ^{1}H-nmr spectra of **1-4** were very similar to one another and to those previously reported for 12-deoxyphorbol esters (9,10). Acetylation of **1-4** with pyridine-Ac_2O at 25° gave no acetylated product suggesting they are presumably 13,20-diester derivatives of 12-deoxyphorbol (**7**).

The mass spectra of **1-4**, which were very informative, supported their structures. All showed three characteristic fragment ion peaks at *m/z* 330, *m/z* 312 and *m/z* 294 followed by a fragmentation pattern similar to that of **6**, below *m/z* 294, but displayed discernible $M^{+\cdot}$ peaks [*m/z* 656 (**1**), *m/z* 684 (2), *m/z* 712



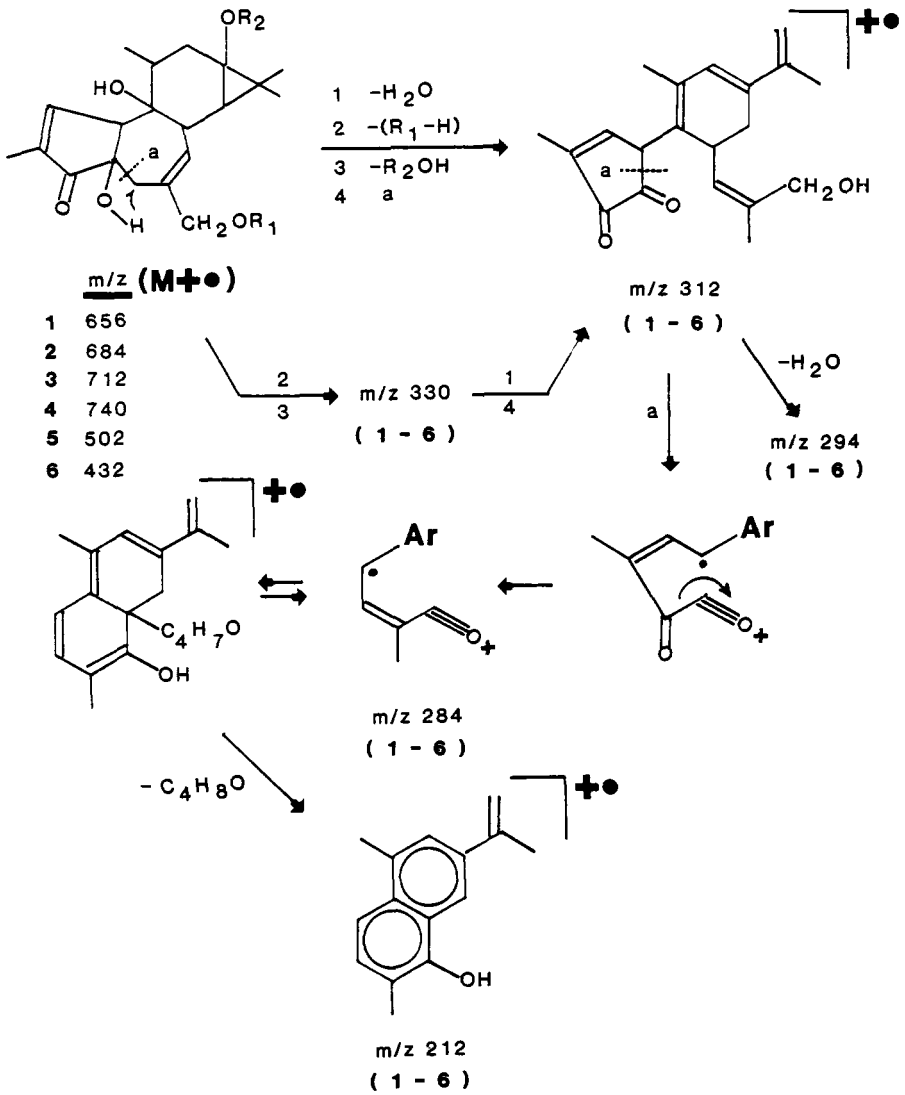
SCHEME I. Isolation of 12-Deoxyphorbol-13,20-diesters (**1–4**) from *Croton californicus*

1 13,20-O-Dideconyl-12-deoxyphorbol.
2 13-O-Decanoyl-20-O-dodecanoyl-12-deoxyphorbol.
3 13-O-Decanoyl-20-O-tetradecanoyl-12-deoxyphorbol.
4 13-O-Decanoyl-20-O-hexadecanoyl-12-deoxyphorbal.

(**3**) and *m/z* 740 (**4**)] differing from one another by 28 mass units, indicating they are homologs. The size of the ester moieties in **1-4** was deduced by substracting *m/z* 346 (mol. wt. of **7**–2H) from $M^{+\cdot}$. The presence of two significant peaks at *m/z* 484 ($M-R_1OH$) and *m/z* 466 ($484-H_2O$) in all four compounds (**1–4**) clearly indicated that $CO(CH_2)_nCH_3$ (n=8, 10, 12 or 14) is one of the ester moieties with the other ester (R_2) being $CO(CH_2)_8CH_3$. Another peak which stands out in all compounds (**1–6**) occurs at *m/z* 212; its genesis, verified by high-resolution exact mass measurement, is rationalized as shown in Scheme II.

Selective hydrolysis of **1-4** with 2% perchloric acid (4), which established the location, size and nature of the ester moieties, gave the same 13-O-decanoyl-12-deoxyphorbol (**5**) moiety but different fatty acid moieties, whose identities were established by direct gc retention time comparison of their esters (prepared by treatment with diazomethane) with authentic samples (Supelco, Inc., Bellefonte, PA). The identity of **5** was established from its ^1H nmr [δ 3.99 (s, 2H, CH_2OH) (11)] and mass spectra; the latter displayed an $M^{+\cdot}$ peak at *m/z* 502 followed by the usual fragmentation pattern after the loss of 172 mass units (*m/z* 330) corresponding to $H+OR_2$ at C-13.

Hydrolysis of **1-4** with methanolic KOH followed by acetylation (pyridine-Ac_2O) of the resulting alcohols gave 12-deoxyphorbol-13,20-diacetate (**6**), identical in all respects (co-tlc, hplc retention time, ir, ^1H-nmr and ms) with an authentic sample.



SCHEME II

EXPERIMENTAL[1]

REVERSE PHASE HPLC.—A solution of the single spot mixture (18 g) dissolved in 7% aqueous methanol (50 ml) was introduced in five equal portions onto the head of a Waters PrepPAK-500/C_{18} cartridge column connected to Waters PrepLC/SYSTEM 500A liquid chromatograph equipped with a refractive index detector. With the flow rate set at 100 ml/min, the column was eluted with 7% aqueous methanol. The collected fractions were combined based on the reverse phase tlc, and the solvent was removed under vacuum. Repetition of the above procedure with the resulting simpler fractions gave compounds **1–4** with few impurities.

Final purification of each of these compounds was performed on a Spectra Physics model 3500B LIQUID CHROMATOGRAPH equipped with a uv detector set at 254 nm and a Valco N60 manual injector with a 10 μl fixed volume loop. With the flow rate set at 2 ml/min, each sample, dissolved in appropriate solvent mixture, was injected at 10 μl/injection. The column (Whatman Magnum 9, 10/50 ODS-2 C_{18}, Clifton, N.J.) was eluted with various methanol-water

[1]The infrared spectra (CCl_4) were obtained on a Beckman IR-33. The ^{1}H-nmr ($CDCl_3$) spectra were determined on a Varian EM-360L with tetramethyl silane as internal standard. Mass spectra (70 eV) were obtained on a Varian GC-MS MAT 311A.

See reference 1 for location of the plant material collected and initial processing and scheme I for the isolation of a tlc single spot complex mixture.

mixtures: 90:10 for **1**, 93:7 for **2**, and 97:3 for **3** and **4**. Compound **5** was purified as above; the column was eluted with 25% aqueous methanol.

Methanol used for hplc was reagent grade filtered through a 0.5 μm type FH Millipore filter. Water, deionized and double-distilled in glass, was filtered (FA filter) as above and degassed by He or N_2 before use.

ACKNOWLEDGMENTS

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