Intl. Chem. Pharm. Med. J. Vol. 1(2), pp.107-111 (2004)

16-HYDROXYINGENOL DITERPENE ESTER FROM THE LATEX OF *EUPHORBIA CAUDUCIFOLIA*

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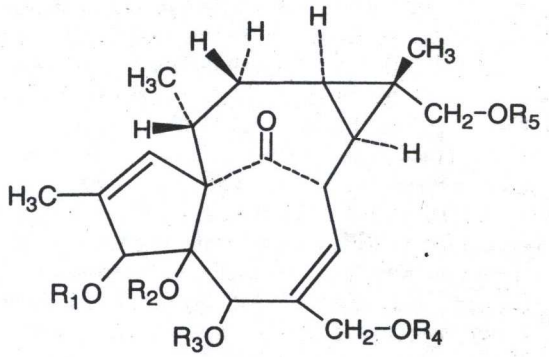
ABSTRACT: A new ingenol ester 16-(2,4,6,8,10-tetradecapentenoyl)-20-palmityl-ingenol was isolated from an acetone – soluble fraction of the latex of *Euphorbia cauducifolia*. This ingenol diterpene ester was identified on the basis of their spectroscopic parameters as well as those of their hydrolytic derivatives and was tentatively identified as 16-(2,4,6,8,10-tetradeca-pentenoyl)-20-palmitylingenol.



1. $R_1=R_2=R_3=H$	R_4= palmityl,	R_5 = 2,4,6,8,10-tetradecapentenoyl
2. $R_1=R_3$=Acetyl	R_2=H, R_4= palmityl	R_5 = 2,4,6,8,10-tetradecapentenoyl
3. $R_1=R_2=R_3=R_4=H$	R_5= 2,4,6,8,10-tetradecapentenoyl	
4. $R_1=R_2=R_3=R_4=R_5=H$		
5. $R_1=R_3=R_4=R_5$=Acetyl	R_2=H	

KEY WORD: *Euphorbia cauducifolia*; Euphorbiaceae; 16-Hydroxyingenol diterpene ester.

INTRODUCTION

Spurges (Euphorbiaceae) are common constituents of many ancient medicine used in the treatment of cancer mentioned in the Greek and Roman medicinal literature (Riddle, 1985). Recent researches have also highlighted the wide spread use of several plants of this family in the treatment of cancerous conditions (Hartwell, 1969). Very recent macro cyclic diterpenes from *E. pubescens* were isolated and evaluated for their in vitro effect on growth of three human cancer cell lines (breast, lungs, and central nervous system) (Claudia *et al.*, 2003).

Euphorbia cauducifolia is very common to Pakistan. Frequently its leaves are used in the treatment of bronchial and intestinal disorders (Kirtikar *et al.* 1991). Roots of this species are antispasmodic ((Jafri, 1966 and Nadkarni, 1976), extract of latex and roots are believed to be anti-tumor (Nadkarni, 1976).

Previous investigations revealed that no remarkable study had been carried out on the latex of this plant. Four inactive diterpenes have been reorted from the root of *E. cauducifolia* (Hussasin, 1977 and NG, 1990). This is the first report on isolation and characterization of biologically active diterpene ester ingenol, which is responsible for toxicity of this species.

During routine testing of column fractions of latex extract of *E. cauducifolia* it was found that polar fractions which have skin irritancy showed pronounced activity at mouse ear, ID_{50} = 0.6 µg/ear and inhibiting activity T/C = 106, with the standard procedure of Hecker *et al.* (1971).

RESULTS AND DISCUSSION

By appropriate combinations of chromatography and multiplicative µ-distribution methods with biological tests for irritant activity, fractionation of the MeOH extract of the latex was accomplished and the highly irritant diterpene ester has been isolated. Later compound **1** was identified as

16-(2,4,6,8,1O-tetradecapentenoyl) 20-palmitylingenol on the basis of spectral studies (UV, IR, MS & NMR), hydrolysis and acetylation (Busch, 1971).

The compound 1 showed irritant activity (lD.so = 0.6 ~g/ear) in model experiments where standard procedure (Hecker *et al.,* 1971) was used on mouse of approximate weight 250g.

Latex hydrophilic traction yielded a colorless oil. Its molecular formula was calculated as Cs0Hs40s by HREIMS observed at *mlz* 812.25341which showed a molecular ion peak 16 a.m.u higher than normal ingenols (Busch 1971). In its IH-NMR spectrum of compound 1 (Cs0Hs40s) was found to exhibit many similarities to that of other hydroxyingenol (Busch 1971). However, an additional two proton ABq, quartet, center at 3.31 ppm and 3.65 ppm as well as D2O-exchangeable singlet at 1.84 ppm as would be expected was absent in the compound 1. Contrarily,. the IH-NMRresonances at 0.84 pmm and 1.16 ppm assigned to the 16 and 17 tertiary methyl groups in the usual ingenols was replaced by more downfield methyl group singlet at 1.25 f,pm in the spectrum. It was, therefore, apparent from its IR, H-NMR, and ElMS data that diterpene moiety of compound 1 contained three methyl groups and two hydroxymethyl groups, and was esterified with one acylate at C-16 and other acylates substituent present at C-3. Close inspection of the IH-NMR spectrum of compound 1 and other ingenols suggested that only feasible positions for the insertion of the additional oxygen function was thought to be in the form of a OH group either at the C-16 or C-17 methyl group since only one methyl was observed in IH-MMR at 1.05 ppm, and a ABq system centered at 4.2 ppm was suggestive of the CH2-O- group of a primary ester, its location on C-16 being rationalized by reference to the molecular models indicated that environment of a C-17 hydroxy methyl would be somewhat more sterically crowded than that of a C-17 hydroxymethyl group, and less likely to account for the facile conversion of hydrolyzed product of 1 to the tetra-acetate that was produced in this study. The positioning in 1 of a primary alcoholic group at C-16 is also favoured on biogenetic grounds.

It is pertinent to point out that Adolf and Hecker have noted the occurrence 16-hydoxyingenol (Busch 1971; Crombe 1955 and Linet *ai.,* 1983). Close observation of the NMR spectrum of 1 revealed that the signal for proton on C-14 had become shifted downfield to a 0.90 ppm frOin its position at 0.73 ppm in the spectrum of 3,5, 16,20tetracetate.

The IR spectrum exhibited characteristic absorptions of OH groups 3545 & 3360 em-I, carbonyl groups 1720, 1735 and 1667 em-I and C=C double bonds 1580, 1550 & 1500 em-I.

In the ElMS spectrum, the prominent fragment peaks at *mlz* 589 (W -COCI4H2sMe (239), 617(M+-CO(CH=CH)s (CH2h CH3 compatible with the presence of a palmityl and 2,4,6,8,1O-tertadecapentenoyl moieties in the molecule. On alkaline hydrolysis, 1 produced palmatic acid and 2,4,6,8,1O-tetradecapentenoic acid which were identified by comparing their TLC, Mass, IR and IH-NMR spectra with authentic samples. The IH-NMR (CDCh) spectrum also showed the signals for protons of double bonds at ppm 5.95 (lH,q, J=1.6 Hz), 6.10 (lH,d, J=3.6 Hz), 7.28 (lH,dd, JI=11.2Hz, h=16Hz), 6.599 (lH,dd, J(=11.0Hz, h=l1Hz), 6,12 (lH,d, J=llHz) and 5.55 (lH, J=9.6Hz) and for the protons on carbon bearing an oxygen atom at ppm 4.73 (lH, d, J=12.8), 4,179 (lH, d,J=12.8 Hz) and 4.43 (lH,s). There were six peaks each for CH3 protons at ppm 1.04 (3H,s), 1.10 (3H,s), 0.96 (3H, d, J=7Hz), 1.85 (3H, d, J=1.7Hz), 0.98 (3H, t, J=7.6Hz) and 0.88 (3H, t, J=7.7 Hz). These spectral data revealed that this compound was a derivative of 16-hydroxyingenQl (Busch 197i; Crombe 1955 and Lin *et aI.,* 1983).

Presence of fragment ions in ElMS spectrum of 1, at *mlz* 330, 312, 292 were suggestive of an ingenane pol)Q1 with the same degree of hydroxylation as 16-hydroxyingenol (Busch 1971; Crombe 1955 and Lin *et aI.,* 1983). In the ElMS spectrum of the compound 1, presence of a peak at *mlz* 310 and absence of peak at' *mlz* 121, confirmed hydroxyingenol (Busch 1971; Crombe 1955 and Lin *et aI.,* 1983). The identity of the parent alcohol became evident from the IH-NMR spectrum of the compound 1, the upfield shift of the 1H doublet for the olefinic proton at C-7, from its usual position at 6.0-6.3 ppm to 5.74 ppm had previously been observed in the spectra of 20-acylingenol (Opferkuch and Hecker, 1974). The structure of the parent alcohol was proposed on the basis of comparative IH-NMR studies of its tetra-acetate with the 3,5,16,20-triacetate of ingenol. It was observed that the signal associated with protons on C-13 and C-14 appeared downfield at 0.9 ppm to 1.4,as compared with 0.73 ppm in the case ofC-13, and C14 protons of ingenol 3,5,16,20-tetra-acetate. Furthermore, the protons of the signals associated with C-8, C-11, and C12 of 16-hydroxyingenol 3,5,16,20-tetraacetate were unaffected by the new acetate, providing evidence for its location on C-16 rather than on C-17. As would be expected, a ~ew signal for two protons at 4.22 ppm ascrible to the protons on C-16, was also evident.

This assignment was further confirmed by the IH and I3C NMR spectra of compound 1, listed in Table 1. The ions in mass at *mlz* 563 & 601 indicated the sequential loss of palmityl (Opferkuch and Hecker, 1974), and 2,4,6,8,10-tetradecapentoyl groups from the molecular ion.

Apart from the signals for the ester groups the IH-NMR . spectrum in CDCh showed two oxymethylene proton signals gem to ester functions as two AB quartets at 0 4.72 (d, J=12.8 Hz) which was assigned'to 20 - CH2, and at 0 4.62, J = 12.64 Hz was attributed to 16 - CH2. The spectrum

also showed 3 methyl groups of diterpene skeleton comprised ofa methyl group at .0.98 ppm doublet wbich was assigned to 18 -CH3, while two methyl singlets at 01.16 and 1.5 were suggested to CH3-17 and CH3-19 groups respectively, its chemical shifts remained unchanged after acetylation (Opferkuch and Hecker, 1974). The downfield shifts of the signals for H-13 and H-14 in 1 with respect to the corresponding signals in ingenol-3, 5, 16, 20-triacetate (Busch 1971) indicates that the additional hydroxy group is at the C-16 of ingenol (Zechmeister *et al.,* 1970). Thus the structure of 1 is analogous to that of 16-hydroxyphorbol [17].In comparision with analogous signals in ingenol - 3,5,20-triacetate (Jia and Ding, 1991), the additional O-acyl group in 1 does not influence the chemical shifts of OH-4B.H-11B,and H-12B, as would be expected for an acylate at the 16 position rather than at the alternative 17 position. The circular dichroism of 1 £: 278 (-0.12), 300 (+0.64).311 nm (+0.715) is in agreement with the data of ingenol-3, 5, 20-triacetate (Jia and Ding, 1991).

All the vicinal relationship of the proton resonances were determined by extensive spin-spin decoupling in C~6 through in lH - lH - COSY experiments.

The 13C-NMR (APT) and DEPT spectra of 1 showed 50 carbon atoms, including 5CH3, 19CH2, 18CH and 8C, of which eight are oxygenated (1 keto, 1 tertiary alcohol, 2 secondary alcohols, 2 primary. alcohols and 2 ester carbonyls) All of the protons bearing carbons were indicated by HETCOR experiments, in addition to the signals of carbonyl at 0 174.33, 173.86 and 177.33. The 13C NMR also revealed five C=C resonances of the esters at 15 134.45, 132.68, *U9.70,* 69.60 and 66.32. However, trom the lHNMR spectrum, the presence of 2 a€yloxy groups, were apparent as ester functional groups. After acetylation under normal condition compound 1 afforded an acetyl derivative 2 which showed an additional two acetoxymethyl singlet at 15 2.07, & 2.3 while the chemical shifts of H-3 and H-5 proton singlet changed trom 15 3.87 H-5 and 5.54 H-3 observed in the spectrum of compound 1 to 15 5.38 and 4.07 respectively. The upfield of H-3 by 15 0.48 on acetylation of OH-5 in compound 1 is due to the anisotropic effect of the acetyl carbonyl group.

Integration of these data, the observed molecular ion peak at *mIz* 812 and the degrees of unsaturation supported the presence of a tetracyclic diterpene moiety C2oH3206 esterified with 2 acids, palmitic acid and 2,4,6,8,10 teradecapentenoic acid. The issue has been resolved therefore the nature of diterpene skeleton and the respective locations of the two esterifying groups.

The coupling constants of IH-1H COSY-45 and lH-13C were nearly identical to those reported for 16-0H ingenane derivatives (Gschwendt and Hecker, 1970; Furstenberger . and flecker, 1977). Thus diterpene moiety of 1 was similar to that of 16-OH ingenol.

Further assignment of the location of acylating groups in 1 was carried out by a IH-13C long range COSY-45 experiments (COLOC). Cross peaks between the carbonyl carbons at 15174.03 C-16, and 173.83 C-20 with protons at 15 2.3 (d, J= 8 Hz) & 5.53 (d, J= 9.5 Hz) respectively, established the location of the ester groups.

The structure of inlfenane 1 followed trom the IH, 13e, lH IH 2D J-resolved, H_13C, 2D-COSY (COLOC) (spectral data table.2) which were similar to those of the 20palmitate-I6-0H-ingenol derivatives (Opferkuch, and Hecker, 1974). In lH-NMR spectrum a second set of the 2,4,6,8,IO-tetradecapentenoic acid appeared and a. CH3 singlt:t was missing. Instead 2 signals forming an ABq system' indicated at C-16 or C-17- (2,4,6,8,10tetradecapenteneoxy derivative of 1.

To confirm this assignment the NOE, experiments were carried out. The NOE effects between the H-17 methyl singlets and the cyclopropane signals required the 2,4 ,6,8 ,1 O-tetradecapenoate group at C-16. The stereochemical assignment of 2,4,6,8,IO-tetradecapentenoic acid was accompiished by well known method adopted by Hecker *et al.* (1977) based on 2D-H NMR experiments and the confirmation of the double bonds as well as cis by comparison of the downfield coupling constants with of 2Z octadecenoic acid (Gschwendt and Hecker, 1970; Furstenberger and Hecker, 1977). Additional evidence for this moiety was obtained by comparing the IR spectrum of 1 and the four geometric isomers of methyldecadieneoate *(Purcellet al.,* 1966). The prominent absorption maxima at 955 and 1000 cm were present only in methyl 2Z, 4E-decadienoate (Purcell *et al.,* 1966).

Taking into account the above facts the structure of compound 1 was proposed as 16-tetradeca-2,4,6,8,1O pentenoyI2~-palmitoyl Ingenol Fig. 1.

EXPERIMENTAL

MPs: uncorrect; *[a]* 25: CHCb; UV: MeOH; FT-IR: film;
HMR: 500 MHz, CDCb, using the FT mode, with TMS as an internal standard; ElMS ca 70 eV; Prep.TLC: silica gel (0.25mm), using 60% H2SO4 as visualizing agent and MelCO to elute zones from plates, solvent system: 1. hexane-C~6-Et20-EtOAc (2:2:1:1); 2. cyclohexane- Et20-EtOAc (1:1:1); 3. CHCb- EtlO (19:1), 4. CHClrMeOH (9:1).

Plant material: Euphorbia cauducifolia latex was collected from Karachi University Campus, Karachi, Pakistan, and was identified by Prof. Dr Nassir Ali. A voucher specimen was deposited in the herbarium of University of Karachi,

Institute *of* Botany Karachi, Pakistan. Latex was collected directly into a flask containing methanol, after one week was evaporated under reduced pressure at 40C.The methanol extract was subjected to MezCO and soluble fraction material evaporate to get crude extract and was named as MezCO soluble fraction which was further treated with EtOAc to afford EtOAc soluble crude then this soluble material after evaporation was partitioned into Pet: ether-MeOH - HzO *(15:10:0.5),* the crude material was dissolved in upper layer *of* this system and extracted with lower layer ,washed with water and dried over anhydrous sodium sulphate and named hyrophillic traction.

Isolation of ingenolie ester: 6.5 g hydrophilic fraction was subjected to silica gel column (60 type 70-230 mesh size E, Merck silica gel for column.chromatography) using 1 as an eluent, tractions 51-109 trom the column during separation exhibited three major zones by TLC when solvent system 1 was used for development. Purification *of* the I which was least polar zone by preparative TLC in solvent 3 yielded 6.0 mg 1 (Rf 0.41).

Characterization of isolate 1: 16-(2,,4,6,8,10 tetradecapentenoyl), 20-palmitylingenol.(6.0mg 0.0056% ,w/w) exhibited the following data:

Spectral Data:
HRMS: 812.25341 [M+],
Cs0Hs40g.
ElMS: (Relative intensity, %,) 70ev, m/z: *812.9~,O.3),* 794(~-18,HzO,0.6), 797(~15,O.9), 776(~-36,2HzO,O.8), 611(~-CO(CH)1O (CHzhCH3,20), 573(~-239, CO(CHz)14CH3.l6), 412,372,310(100), 294(30), 197(47), 83(30).
UV (MeOH): An"",; 192, 210, 302,5nm (18,000,14000,27 700);
IR (CHzCh): 3678, 3567, 3517, 3515, 3423,1716,1627, 1608 cm-l .
H-NMR (CDCh, 500MHz): ppm, 7.85 (I-H), 6.8-5.7 (7H), H-3; 5.63, H-8 1; 4.3, CHr20:4.31, H-5:4.04, CH319:1.8, CH3-1(3, CH3-17:1.08, 1.05, 2-0H (exchangeable) : 3.36ppm.
13CNMR(CDCh, 75.5MHz): ppm 131.65C/l, *163.26C/2,* 82.8OC/3, 84.73C14, 74,95C/5,136.60 Cl6, 128.38 *C/8,* 205. 17C/9,71.97C110,37.67 *C/ll,35.12 C/12,* 69.08 Cl13,28.39 *C/14,* 30.42 *C/15,* 66.49 *C/16,* 16.71 Cl17,18.19 *C/18,* 15.52 *C/19,66.34* Cl20.
Palmitate: 174.33C11, *34.50CI2,* 23.09C13, 29.67C/4, 29.67C/5,
29 .67C/6,29 .67Cn ,29 .60C/8 ,29 .60Cl9 ,29 ,32CIl 0,29 .29C/l
1,29.15C/12,29.47CIl3,31.91C114,22.70C/15,14.lOCl16.
2,4,6,8,IOTetradecapentenoate: 173.86 Cll, 134.45 Cl2, *132.67C/3, 129.70C/4,* 129.67 *C/5,* 129.65 *C/6,129.65 cn,* 129.64 *C/8,* 129.56 *C/9,* 129.50 *CIlO,* 66,56 Clll, 29.15 Cl12, 29.43 *CIl3,* 31.93 Cl14, 22.70Cl15, 14.10 *C1l6.*
Pal1ial hydrolysis of compound 1: Compound 1 (2.5mg) was hydrolyzed with O.IM KOH in MeOH for 20 minutes at room temperature. The h}drolysis product obtained, 20-palmitylingenol (2, 1.4 mg), on purification by preparative TLC in solvent system 1 (Rf 0.26) was characterized as follows: resins; [11]D 25 - 23.6 (CHCI3, c, 0.3).
IR (KBr) 'Umax: cm-l 34 23, (OH), 1723, 1223 (ester), 1673, 3045 (C=C) 739;
EI-MS: m/z (reI. int., %): 602 ~, 3), 584 (M+-HzO, 6,)+, 587 (~ -Me, 7), 566 (~-2HzO,8), 363 (16),255 (28),213 (47), 83 *(100).* lH-NMR(CDCh) ppm; 5.75 (lH,s, H-l), 5.62,(IH,s H-3), 4.06,(IH,s H-5), 6.90 ,(IH,s,H-7), 4.20, (IH,m,H-8),(1H,m ,H-ll),2.34(IH, m,H-12), 0.68(IH, m,H-13), 0.68,(IH, m, H-14), 4.48,(2H,ABq J=12.2 Hz,CHr
H-16), 1.15(3H, CH3-17), 0.98, (3H, CH3-18), 1.83(3, CH319), 4.72(2H, CHz-20), 2.30 (3H, t, J=8 Hz, CH3-2), 1.25(2H, br. s, CHr3, CHz-15), 0.88 (t, J=7.5Hz, CH3-16).

Compound 16-hydroxyingenol-3, 5, 16, 20-tetraacetate was produced and identified (mp, EI-MS, co- TLC), after work-up by direct comparison with a sample *of* this compound obtained in earlier work on *E. ingens* [27]. MS: Parent ion at m/z= 520, NMR, 2.3 ppm, 4-CH3CO.

Transesterification of 1: Sodium methoxide solution in methanol (10 ml, 0.005M) was added to 1 (5.mg). After 20 hours, phosphate buffer was (10 ml; pH 6.8) was added. After removal *of* the majority *of* methanol under reduced pressure, the residue was taken in ethyl acetate and washed with water. Evaporation *of* the solvent gave polyhydroxyingenol V which was purified by TLC in dichloromethanel acetone (4/1) to yield 3.7mg *(Rf* = 0.11) MS: Parent ion m/z=/362.

IR (CHzCh): 3600,3550.3445,3437,3367, (OH), 1605, (CO) 1550 (C=C), lHNMR;3-H:3.82 ppm.

Catalytic hydogenation of polyhydroxyingenol (V): 18 mg alcohol (V) was dissolved in ethyl acetate 5ml was hydrogenated with hydrogen gas in 10 % Pd Icharcoal (8mg) for five hours at room temperature. After filtration and evaporation *of* the solvent, the remaining residue was purified by TLC in dicHloromethane/acetone (311), *Rf* = 0.36. MS:Parent ion at m/e 364.

MS:Parent ion m/z= 364.tHNMR: 6-H: 1.54(IH,m), 7-2H: 1.86(2H, m,)

Acetylation of polyhydroyingenol (V): Compound V 3.6mg was dissolved in acetic anhydride (0.3ml) and pyridine 0.6ml and the reaction mixture left at room temperature overnight. Crushed ice was added and the mixture extracted with ethyl acetate. After removal *of* the solvent afforded crude product which was purified by TLC in benzene/ethyl acetate *3/1* to yield tetra-acetate 3,5,16,20-ingenol 3.3mg Rf=0.42.

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

	Irritant Dose 5% (µg/5ul)			
Compound	4hr	S*	24hr	S*
1.....	0.04	1.24	0.07	1.28
Phorbol-12, 13-dibenzoate	>100	-	>	-
Phorbo-12-tetradecanoate	0.60	1.29	0.83	1.20
Phorbol-13-acetate.....	0.04	1.25	0.05	1.26

*S= Standard Deviation.

MS: Parent ion m/z 520; ^{1}H-NMR: 6.25 (1H, m, H-7), 6.1(1H, s, H-1), 5.40 (1H, bs, H-5), 4.20 (1H, m, H-8), 4.4(2H, ABq, JAB = 12.7Hz), 5.00 (1.77 3H, d), 4.29(2H, s, CH_2-16), 1.15(3H, s, CH_3-17), and 2.2-2.5 (12H, s, 4*CH_3CO).

*p-Bromobenzoylester of 1:*A solution of p-bromobenzoyl chloride 10 mg in benzene 2 ml was added into a solution of 1 (10 mg) in dry pyridine 0.5ml and the mixture was left for 3 hours at room temperature. Crushed ice was added followed by extraction with ethyl acetate. The organic layer washed with 1M HCl, 5% aqueous $KHCO_3$, then with water, dried over anhydrous sodium sulphate and concentrated. The crude product was purified by TLC in benzene/ethyl acetate (8/1); Rf = 0.44, to yield 6mg of 5-p-bromobenzoate of 1. MS: Parent ion. m/z = 995, NMR: 3-H: 5.56 (d, J=5Hz), 5-H: 5.5(1H, d, J = 5.5Hz), 20-2H, 4.45(ABq, J = 12.5 Hz)

Identification of un-saturated fatty acids in 16-hydroxyingenol: The compound 1 was identified as diacylates of 16-hydroxyingenol carrying conjugated double bonds in the carboxylic acids, as revealed by the absorption maxima at 310 and 352 nm in the UV spectra of the compound 1.These acids were identified by following standard procedure adopted by Hecker *et al.* (1974). Upon treatment with Me_2CO/p-toluene sulphonic acid hydrates isopropylidene derivatives were formed. Through base catalyzed trans-esterification was carried out to yield O-isopropylidine ingenol and un-saturated carboxylic acid methyl ester.after catalytic hydrogenation later was identified by GC analysis were identified as palmitatic and 2,4,6,8,10- tetradecapentenoic acid.

Biological Testing: Irritant dose 50% (ID50) determination of acetone soluble fractions (diterpene esters) were performed by the method of Hecker *et al.* (1971) except that readings were made at both 4 hrs and 24 hrs after samples were applied. Assays were carried out using male Swiss Webster mice, maintained at 20 C 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 ID50 and Standard deviations are shown with of 1 in table 1.

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Manuscript submitted on May 15, 2004
Accepted for publication June, 9, 2004