Table V. Isoflavone Content of Clark[a] Soybeans Grown in Urbana, IL, in Different Years (mg/100 g)

isoflavone	1975	1976	1978	1979	LSD[b]
daidzin	75.4	124.4	98.7	82.6	4.3
glycitin 7-β-glucoside	19.9	22.8	25.5	23.5	2.3
genistin	153.2	210.4	157.4	135.4	7.8
daidzein	2.3	0.8	1.1	1.2	1.5
glycitein	3.0	3.2	1.9	2.0	1.3
genistein	0.7	0.9	0.2	0.6	0.7
total	254.7	362.5	284.9	245.2	14.7

[a] Clark variety = Lincoln[2] × Richland. [b] Least significant difference at 0.05 probability level, based on sample variation.

larger amounts of the isoflavones than the Hardin and Amcor, even though the four samples were grown the same year in the same location. Genistin appears to vary considerably between samples.

Further investigations into different varieties were conducted. Two varieties, Hardin and Corsoy-79, were grown the same year in four different locations in Illinois. The results of the eight samples for isoflavones and isoflavone glucosides are shown in Table IV.

The concentration of the isoflavones and isoflavone glucosides vary from variety to variety, and there are also differences when the same variety is grown in different locations. Significant variety–location interactions were observed for daidzin, glycitin 7-β-glucoside, genistin, and the total isoflavones. Varietal differences at Girard and Urbana differ from the varietal differences at Pontiac and DeKalb. These results may indicate adverse growing conditions in different locales in 1980, which was a dry year in Illinois.

Table V shows the amounts of isoflavones found in Clark soybeans when grown in Urbana, IL, in different years. Significant variation among years suggests that unknown climatic and environmental factors contribute to variation in isoflavones and isoflavone glucosides.

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Three New Ingenane Derivatives from the Latex of *Euphorbia canariensis* L.

Lee-Juian Lin and A. Douglas Kinghorn*

Three new ingenane esters, 3-*O*-acetyl-16-*O*-benzoyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (1), 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-benzoyl-16-hydroxyingenol (2), and 3-*O*-acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (3), were isolated from the latex of *Euphorbia canariensis* L. by using droplet countercurrent chromatography. The structures of these skin-irritant compounds were established through the interpretation of spectroscopic data. *E. canariensis* is sold in the United States as an ornamental plant and is currently under investigation for possible cultivation as a renewable energy source. Constituents 1–3 represent a health hazard for persons who contact the latex of this species with the skin or eyes.

Euphorbia canariensis L. (Euphorbiaceae) has recently been suggested as a candidate plant for cultivation in semiarid regions to produce fuel, since its latex is rich in isoprenoids (Calvin et al., 1982). This species, although native to the Canary Islands, is now available for purchase from nurseries in the United States as an ornamental houseplant. In previous work, *E. canariensis* latex has been shown to evoke severe skin inflammation in mice (Kinghorn and Evans, 1975) and, on repeated administration subsequent to a subcarcinogenic dose of 7,12-dimethylbenz[a]anthracene, has produced pronounced tumor-promoting effects on mouse skin (Roe and Peirce, 1961).

A number of constituents of *E. canariensis* latex has been investigated, including inositol and a phenol oxidase

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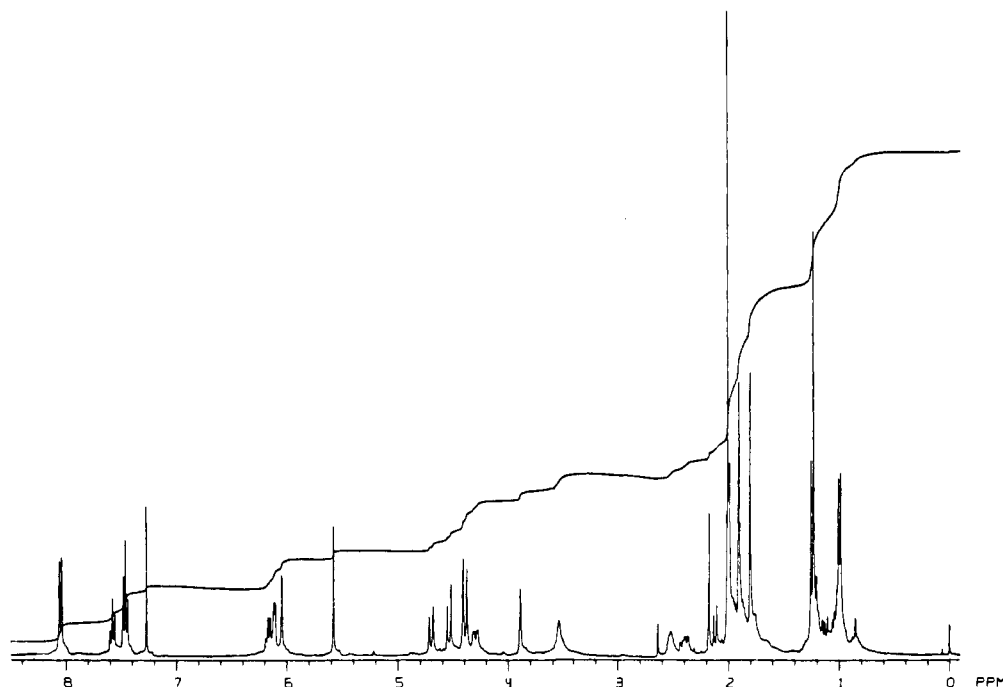
Figure 1. ^{1}H NMR of 3-*O*-acetyl-16-*O*-benzoyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (1).

enzyme (Santos Ruiz and Santos Merino, 1949), sterols (González González and Calero, 1949), tannins (García Martin, 1952), and D-glucaric acid (Winsnes, 1972). However, while it is now well established that diterpene esters of the tigliane, ingenane, and daphnane types occur as the skin-irritant and tumor-promoting constituents of plants in the genus *Euphorbia* (Hecker, 1978; Kinghorn, 1979), the precise nature of the toxic principles of *E. canariensis* latex has not been determined. Phytochemical studies toward this end are the subject of this investigation.

In this communication, we report the isolation and characterization of three skin-irritant ingenane ester constituents (1–3) of the latex of *E. canariensis*. These compounds were isolated by a rapid procedure involving droplet countercurrent chromatography (DCCC).

RESULTS AND DISCUSSION

Compound 1, the most abundant of the three skin-ir-
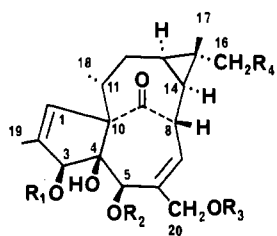
1 R_1 = CH_3CO; R_2 = H; R_3 = ⟨CO⟩ ; R_4 = C_6H_5OCO

2 R_1 = ⟨CO⟩ ; R_2 = R_3 = H; R_4 = C_6H_5OCO

3 R_1 = CH_3CO; R_2 = R_4 = H; R_3 = ⟨CO⟩

4 R_1 = R_2 = R_3 = CH_3CO; R_4 = CH_3COO

5 R_1 = R_2 = R_3 = H; R_4 = C_6H_5OCO

6 R_1 = R_2 = H; R_3 = ⟨CO⟩ ; R_4 = C_6H_5OCO

7 R_1 = R_2 = R_3 = CH_3CO; R_4 = H

8 R_1 = R_2 = R_4 = H; R_3 = ⟨CO⟩

ritant constituents of the *E. canariensis* latex sample

studied in this investigation, exhibited spectral (IR, UV, ^{1}H NMR, MS) properties consistent with being an ester of 16-hydroxyingenol (Opferkuch and Hecker, 1974; Adolf and Hecker, 1975; Upadhyay and Hecker, 1975). Following hydrolysis with 0.5 M methanolic KOH and acetylation, the known compound 16-hydroxyingenol-3,5,16,20-tetra-acetate (4) was obtained. The molecular formula of 1 was assigned as $C_{34}H_{40}O_9$ by high-resolution mass spectrometry, and other MS observations indicated the presence of three ester substituents in this molecule with molecular weights of 60, 122, and 100, respectively. In the ^{1}H NMR spectrum of 1, chemical shifts were observed in accord with the occurrence of acetic, benzoic, and (*Z*)-2-methyl-2-butenoic (angelic) acid moieties (Table I; Figure 1). In the latter case, multiplets assignable for the vinyl methyl groups occurred at δ 1.99 and 1.91, while the chemical shift of the olefinic proton of this substituent was observed at δ 6.16. The presence of either of the isomeric (*E*)-2-methyl-2-butenoic (tiglic) or 3-methyl-2-butenoic (senecioic) acid substituents units in 1 could therefore be precluded, since the vinyl protons of these compounds absorb at about δ 6.95 and 5.63, respectively (Bohlmann et al., 1977). Further, on the basis of comparison of the ^{1}H NMR spectrum of 1 with those of other ingenol derivatives (Opferkuch and Hecker, 1974; Hirota et al., 1980; Opferkuch et al., 1981), it was possible to assign the positions of ester substitution as occurring at C-3, C-16, and C-20, owing to the respective observation of resonances at δ 5.58 (sharp singlet), 4.39 (doublet), and 4.70 and 4.54 (AB quartet) (Table I; Figure 1).

For determination of the relative position of ester substitution in 1, selective hydrolysis experiments were carried out. Hydrolysis with 0.5 M KOH in methanol produced in low yield a 16-hydroxyingenol monoester (5), with a molecular weight of 468, incorporating a benzoate functionality. Upfield shifts were observed in the ^{1}H NMR spectrum of 5, relative to that of 1, for the C-3 methine and the C-20 methylene protons (Table I), so it could be concluded that 5 was 16-*O*-benzoyl-16-hydroxyingenol and that the benzoate unit in 1 was attached at C-16. Alkaline hydrolysis of 1 under milder conditions with 0.1 M methanolic KOH resulted in the formation of the 16-hydroxyingenol diester, 6, which, on analysis of its ^{1}H

Table I.   ¹H NMR Data for Ingenane Derivatives 1-8[a]

	1[b]	2[c]	3[b]	4[c]	5[c]	6[c]	7[c]	8[c]
H-1	6.04 d (1.5)	6.03 d (1.5)	6.05 d (1.5)	6.08 d (1.5)	5.88 d (1.8)	5.91 d (1.7)	6.07 d (1.5)	5.93 d (1.1)
H-3	5.58 s	5.59 s	5.56 s	4.96 s	4.40 s	4.39 s	4.97 s	4.42 s
H-5	3.89 br s	4.06 br s	3.90 br s	5.38 br s	3.80 br s	3.69 br s	5.38 br s	3.69 br s
H-7	6.11 d (4.0)	6.07 m	6.13 d (4.6)	6.25 d (7.0)	5.99 d (7.0)	6.08 m	6.24 d (5.0)	6.02 d (4.7)
H-8	4.30 dd (12.1, 4.2)	4.17 m	4.10 dd (11.9, 4.2)	[d]	[d]	4.22 m	4.25 m	4.10 m
CH₂-16	4.39 d (11.9)	4.46 br s		4.18 br s	4.52 br s	4.51 br s		
Me-16			1.09 s[e]				1.06 s	1.06 s[e]
Me-17	1.23 s	1.24 s	1.06 s[e]	1.14 s	1.25 s	1.24 s	1.06 s	1.11 s[e]
Me-18	1.00 d (7.0)	0.99 d (7.4)	0.98 d (7.1)	0.99 d (7.4)	0.95 d (7.3)	0.98 d (6.6)	0.99 d (7.0)	0.97 d (7.2)
Me-19	1.81 d (1.1)	1.80 d (1.1)	1.81 d (1.2)	1.75 br s	1.82 d (1.1)	1.84 br s	1.75 br s	1.83 br s
CH₂-20	4.70, 4.54 AB q (J_{AB} = 12.2)	4.06 s	4.77, 4.49 AB q (J_{AB} = 12.5)	4.61, 4.15 AB q (J_{AB} = 13.0)	4.05 s	4.70, 4.52 AB q (J_{AB} = 12.0)	4.61, 4.15 AB q (J_{AB} = 13.0)	4.78, 4.59 AB q (J_{AB} = 12.0)
-OCOMe	2.01 s		2.07 s	2.22 s, 2.11 s, 2.07 s, 2.00 s			2.21 s, 2.12 s, 1.99 s	
-OCOC₆H₅	7.44-8.06 m	7.41-8.14 m			7.42-8.13 m	7.40-8.10 m		
-OCOC(CH₃)=CHCH₃	6.16 m, 1.99 m, 1.91 m	6.07 m, 2.06 m, 1.93 m	6.18 m, 2.01 m, 1.93 m			6.08 m, 1.95 m, 1.84 m		6.11 m, 1.87 m, 1.85 m

[a] Spectra were run in CDCl₃ and Me₄Si was used as the internal standard.   Values are recorded in parts per million relative to Me₄Si; coupling constants (hertz) are quoted in parentheses.   Multiplicity is designated as follows: s, singlet; d, doublet; q, quartet; m, multiplet whose center is given; AB, AB system; br, broad.   [b] Spectrum determined at 360 MHz.   [c] Spectrum determined at 60 MHz.   [d] Obscured by other signals.   [e] Assignment interchangeable.

NMR and mass spectra, was shown to possess a benzoate function at C-16 and a (Z)-2-methyl-2-butenoate function at C-20. Hirota et al. (1980) have shown previously that ingenol 3-esters are more susceptible to alkaline hydrolysis than ingenol 20-esters. Therefore, compound 1 was assigned as 3-O-acetyl-16-O-benzoyl-20-O-[(Z)-2-methyl-2-butenoyl]-16-hydroxyingenol and is the first triester of this parent diterpene alcohol to be characterized as a natural product.

Analysis of the ¹H NMR (Table I) and mass spectra of compound 2 suggested that it was a 16-hydroxyingenol diester, containing (Z)-2-methyl-2-butenoyl and benzoate moieties, substituted at C-3 and C-16. On hydrolysis with 0.5 M KOH in methanol and acetylation, 16-hydroxyingenol-3,5,16,20-tetraacetate (4) was produced. It was not possible to prove the relative positions of the two ester substituents by selective hydrolysis, due to the paucity of 2 isolated. However, it may be rationalized that the benzoate group was attached to C-16 in 2, as in 1, due to the observation in their ¹H NMR spectra of a common deshielding effect induced by the aromaticity at C-16 on the C-17 methyl proton chemical shift. This phenomenon resulted in a slight downfield shift of this signal to δ 1.23 in 1 and 2 (Table I) compared with ca. δ 1.14 in the ¹H NMR spectra of 4 and another 16-hydroxyingenol ester containing a C-16 aliphatic ester substituent (Opfurkuch and Hecker, 1974). Compound 2 was thus assigned the structure 3-O-[(Z)-2-methyl-2-butenoyl]-16-O-benzoyl-16-hydroxyingenol.

The ¹H NMR (Table I; Figure 2) and mass spectral characteristics of compound 3 showed evidence of this compound being an ingenol 3,20-diester, and ingenol-3,5,20-triacetate (7) was produced on hydrolysis with 0.5 M methanolic KOH and acetylation. Mild hydrolysis with 0.1 M KOH resulted in the generation of 20-O-[(Z)-2-methyl-2-butenoyl]ingenol (8) and acetic acid. Compound 3 was therefore identified as 3-O-acetyl-20-O-[(Z)-2-methyl-2-butenoyl]ingenol. Recently, Abo and Evans (1982) isolated from another Euphorbia species a positional isomer of 3, with the same acid units substituted in an opposite fashion, although the tentative structure proposed for their isolate was not verified by selective hydrolysis.

We and other groups have experienced difficulties in the isolation of naturally occurring esters of ingenol from Euphorbia species, since these compounds tend to occur in complex mixtures that are difficult to separate and may be unstable to conventional phytochemical techniques of fractionation (Adolf and Hecker, 1975; Upadhyay and Hecker, 1975; Hickey et al., 1981; Abo and Evans, 1982). Droplet countercurrent chromatography has been applied in our laboratory earlier for the separation of the tigliane derivatives phorbol and 4α-phorbol from croton oil (Marshall and Kinghorn, 1981), and the modified system described in this paper presents a substantial improvement over existing methods for the rapid isolation, without apparent decomposition leading to artifact formation, of biologically active ingenane derivatives.

In view of the potent activity displayed by ingenol-3,5,20-triacetate (7) (Hickey et al., 1981), the presence of the skin-irritant compounds 1-3 in E. canariensis latex in a combined yield of about 0.15% w/w would be expected to cause severe and prolonged inflammation if contacted with human skin. In addition, it may be pointed out that 1-3 were obtained from the same E. canariensis specimen as was the latex found by Roe and Peirce (1961) to exhibit tumor-promoting activity on mouse skin (Keesing, 1982). Therefore, in view of the known activity
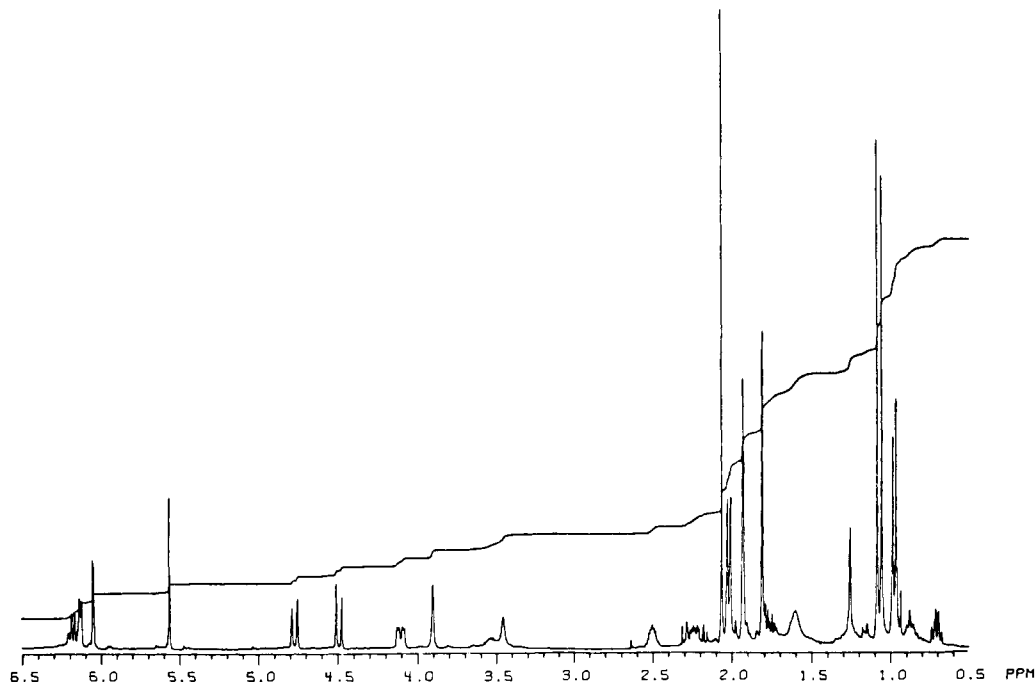
Three New Ingenane Derivatives from *E. canariensis*

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Figure 2. ¹H NMR spectrum of 3-*O*-acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**3**).

of certain ingenol esters as mouse-skin tumor promoters (Hecker, 1978), one or more of constituents **1–3** may be expected to be active in this respect.

EXPERIMENTAL SECTION

The melting point was measured on a Kofler apparatus and is uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV and IR spectra were recorded, respectively, with a Beckman Model DB-G grating spectrophotometer (recorded in EtOH) and a Beckman IR-18A spectrophotometer (recorded in CHCl₃, with polystyrene calibration at 1601 cm⁻¹). ¹H NMR spectra were recorded in CDCl₃, by using Me₄Si as the internal standard, on either a Nicolet NT-360 spectrometer (360 MHz) or a Varian T-60A instrument, with a Nicolet TT-7 Fourier transform attachment (60 MHz). Low-resolution mass spectra were obtained on a Varian MAT 112S instrument (ca. 20 eV), and high-resolution mass spectra were recorded by peak matching on an AEI MS-902 instrument (70 eV). Droplet countercurrent chromatography (DCCC) was performed at room temperature on a Model A instrument (Tokyo Rikakikai, Tokyo, Japan). Analytical and preparative thin-layer chromatography (TLC) was conducted on silica gel GHLF (Analtech Inc., Newark, DE), with 250-μm thick layers, using four solvent systems, viz., hexane–toluene–diethyl ether–ethyl acetate (2:2:1:1, solvent 1), cyclohexane–diethyl ether–ethyl acetate (1:1:1, solvent 2), chloroform–diethyl ether (19:1, solvent 3), and chloroform–methanol (9:1, solvent 4). TLC plates were visualized by using 70% w/v sulfuric acid (110 °C, 10 min).

Plant Material. Latex of *E. canariensis* L. (Euphorbiaceae) was collected into methanol in June 1978. This authenticated plant material (catalog no. 687.32.68701) was obtained at the Royal Botanic Gardens, Kew, Richmond, U.K.

Extraction and Isolation of Diterpene Esters. Dried latex (10.3 g) was exhaustively extracted with acetone, and the residue (4.1 g) was partitioned between hexane (60 mL) and methanol–water (17:3, 2 × 25 mL). The combined polar layers were adjusted to a 1:1 v/v methanol–water ratio, by the addition of 28 mL of water, and were then extracted with 2 × 30 mL of methylene chloride. On

drying, the residue from the less polar solvent (0.44 g) was subjected to fractionation by DCCC, by using a saturated mixture of hexane–ether–1-propanol–ethanol–water (7:16:6:10:8) as the developing solvent, with the upper phase as the mobile phase. The solute was dissolved in 5 mL of the mobile phase and introduced into a 10-mL sample chamber. Ascending development was employed at a pressure of 2–4 kg/cm², and fractions (120 drops each) were collected in an automatic fraction collector.

Fractions 48–50 from the DCCC separation were purified by sequential preparative TLC in solvent 1 (R_f 0.25) and solvent 2 (R_f 0.52) to yield 6.4 mg of compound 1. DCCC fractions 51–55 were purified by preparative TLC in solvents 2 and 3 (R_f 0.55 and 0.17, respectively) to produce 3.6 mg of compound **3**. Preparative TLC of DCCC fractions 56–66 in solvent 2 (R_f 0.26) provided pure compound 2 (1.4 mg).

This isolation procedure was repeated on a further 17.3 g of dried latex, to produce, respectively, 28.8, 2.7, and 11.0 mg of compounds **1, 2,** and **3**. Since **1–3** exhibited similar TLC migration characteristics, both when in the crude *E. canariensis* latex acetone extract and after isolation, there was no evidence of compound decomposition during this DCCC–preparative TLC isolation procedure.

Characterization of Ingenane Derivatives 1–3. 3-*O*-Acetyl-16-*O*-benzoyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**1**, 0.104% w/w) exhibited the following physical, chromatographic, and spectroscopic properties: resin, [α]²⁴_D + 15.0° (c 0.05, CHCl₃); UV λ_max 225 nm (log ε 4.13), 272 (2.86), 280 (2.78); IR ν_max 3530, 2950, 1710, 1630, 1440, 1370, 1260 cm⁻¹; ¹H NMR (360 MHz) see Table I and Figure 1; MS m/z (rel intensity) 592 (M⁺·, 2), 432 (9), 414 (6), 370 (5), 352 (4), 327 (3), 310 (21), 292 (16), 282 (18), 264 (12), 189 (20), 151 (40), 133 (17), 122 (26), 105 (72), 83 (100); mass measurement, found 592.2678, calculated for C₃₄H₄₀O₉ 592.2670; R_f 0.25 (solvent 1).

3-*O*-[(*Z*)-2-Methyl-2-butenoyl]-16-*O*-benzoyl-16-hydroxyingenol (**2**, 0.0098% w/w) exhibited the following physical, chromatographic, and spectral properties: resin; [α]²⁴_D + 1.30° (c 0.09, CHCl₃); UV λ_max 225 nm (log ε 4.17), 270 (2.99), 280 (2.88); IR ν_max 3460, 2920, 1720, 1261, 1017 cm⁻¹; ¹H NMR (60 MHz) see Table I; MS m/z (rel inten-

sity) 550 (M$^+$·, 0.5), 532 (0.5), 472 (1), 450 (2), 432 (4), 414 (2), 328 (3), 310 (9), 292 (7), 282 (10), 187 (10), 160 (15), 151 (21), 133 (12), 123 (14), 122 (19), 105 (54), 83 (100); mass measurement, found 550.2561, calculated for C$_{32}$-H$_{38}$O$_8$ 550.2564; R_f 0.06 (solvent 1).

3-*O*-Acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**3**, 0.04% w/w) exhibited the following physical, chromatographic, and spectroscopic properties; resin; [α]^{24}$_D$ +18.0° (*c* 0.07, CHCl$_3$); UV λ$_{max}$ 218 nm (log ε 4.37); IR ν$_{max}$ 3530, 2930, 1710, 1630, 1440, 1365, 1220 cm^{-1}; ^{1}H NMR (360 MHz) see Table I and Figure 2; MS *m/z* (rel intensity) 472 (M$^+$·, 0.3), 454 (1), 412 (1), 394 (1), 372 (5), 354 (3), 330 (2), 312 (23), 294 (20), 284 (17), 251 (13), 221 (14), 188 (19), 162 (22), 153 (27), 151 (26), 135 (28), 122 (82), 121 (51), 83 (100); mass measurement, found 472.2465, calculated for C$_{27}$-H$_{36}$O$_7$ 472.2459; R_f 0.32 (solvent 1).

Hydrolysis and Acetylation of 1. Compound **1** (9.0 mg) was hydrolyzed with 0.5 M KOH in dry MeOH for 30 min at room temperature. Two products were observed on TLC analysis, with the more polar compound (3 mg) purified by preparative TLC in solvent 4 (R_f 0.19) and acetylated in C$_5$H$_5$N–(Ac)$_2$O (4:1, 0.5 mL) for 1 h at 100 °C. Extraction into CHCl$_3$ and preparative TLC in solvent 3 (R_f 0.11) afforded the resinous 16-hydroxyingenol-3,5,16,20-tetraacetate (**4**, 3.0 mg): [α]^{24}$_D$ -24.3° (*c* 0.05, CHCl$_3$); R_f 0.13 (solvent 1). This derivative exhibited closely comparable spectral data (UV, IR, ^{1}H NMR, MS) to literature values for 16-hydroxyingenol-3,5,16,20-tetraacetate (**4**) (Opferkuch and Hecker, 1974; Upadhyay and Hecker, 1975). The ^{1}H NMR data obtained for **4** are shown in Table I.

Purification of the less polar compound from the above hydrolysis procedure by preparative TLC in solvent 4 (R_f 0.45) afforded 16-*O*-benzoyl-16-hydroxyingenol (**5**, 3.4 mg), which exhibited the following data: resin; [α]^{24}$_D$ +3.4° (*c* 0.09, CHCl$_3$); UV λ$_{max}$ 228 nm (log ε 4.05), 272 (2.90), 280 (2.78); IR ν$_{max}$ 3430, 3020, 2975, 2950, 1720, 1590, 1390, 1320, 1280 cm^{-1}; ^{1}H NMR (60 MHz) see Table I; MS *m/z* (rel intensity) 468 (M$^+$·, 2), 450 (3), 432 (2), 414 (1), 386 (2), 346 (2), 328 (10), 310 (10), 292 (7), 264 (19), 223 (10), 187 (12), 177 (13), 161 (16), 151 (24), 123 (38), 122 (25), 105 (100); R_f 0.01 (solvent 1).

Mild Hydrolysis of 1. Compound **1** (2.5 mg) was partially hydrolyzed with 0.1 M KOH in dry MeOH for 5 min at room temperature. The less polar and major product of the two derivatives of **1** obtained [**6**, 16-*O*-benzoyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol, 1.5 mg], when extracted into CHCl$_3$ and purified by preparative TLC in solvent 2 (R_f 0.34), was found to exhibit the following data: resin; [α]^{24}$_D$ -19.9° (*c* 0.06, CHCl$_3$); UV λ$_{max}$ 225 nm (log ε 4.24), 274 (2.95), 282 (2.91); IR ν$_{max}$ 3460, 2920, 1720, 1700, 1270, 1151, 1018 cm^{-1}; ^{1}H NMR (60 MHz) see table I; MS *m/z* (rel intensity) 550 (M$^+$·, 3), 532 (2), 514 (2), 450 (3), 432 (5), 414 (3), 386 (4), 310 (13), 292 (8), 282 (12), 160 (22), 151 (19), 147 (18), 133 (12), 122 (119), 105 (64), 83 (100); R_f 0.09 (solvent 1).

Hydrolysis and Acetylation of 2. Compound **2** (0.5 mg) was completely hydrolyzed and acetylated as described for compound **1**, and the product, on purification, shown to be 16-hydroxyingenol-3,5,16,20-tetraacetate (**4**) by its MS and TLC characteristics.

Hydrolysis and Acetylation of 3. Compound **3** (3.0 mg) was hydrolyzed with 0.5 M KOH in MeOH and acetylated in the same manner as described for compound **1**. The acetylated product was extracted into CHCl$_3$ and purified by preparative TLC in solvent 1 (R_f 0.34). This isolate (ingenol-3,5,20-triacetate, **7**, 2 mg) was crystallized from acetone [mp 192–195 °C and [α]^{24}$_D$ +39.0° (*c* 0.10,

MeOH) [lit. mp 201–203 °C and [α]^{18}$_D$ +73.0° (*c* 0.31, MeOH) (Hirota et al., 1980)]], exhibited closely comparable spectral data (UV, IR, ^{1}H NMR, MS) to literature values (Hirota et al., 1980; Hickey et al., 1981; Opferkuch et al., 1981), and was indistinguishable from an authentic sample (^{1}H NMR, MS, TLC) obtained previously in our laboratory. The ^{1}H NMR data obtained for **7** are presented in Table I.

Mild Hydrolysis of 3. Compound **3** (2.9 mg) was hydrolyzed with 0.1 M KOH in dry MeOH for 10 min at room temperature. The less polar of two hydrolyzed products [20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol, **8**, 1 mg] was extracted into CHCl$_3$ and purified by preparative TLC in solvent 2 (R_f 0.43) and exhibited the following data: resin; UV λ$_{max}$ 212 nm (log ε 4.02); ^{1}H NMR (60 MHz) see Table I; MS *m/z* (rel intensity) 430 (M$^+$·, 0.5), 412 (2), 394 (1), 330 (3), 312 (12), 294 (8), 284 (7), 241 (8), 162 (21), 151 (21), 147 (18), 136 (17), 135 (20), 122 (23), 109 (13) 97 (14), 83 (100); R_f 0.14 (solvent 1).

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