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THE DERMATITIS-PRODUCING CONSTITUENTS OF *EUPHORBIA HERMENTIANA* LATEX[1,2]

LEE-JUIAN LIN, GARY T. MARSHALL, and A. DOUGLAS KINGHORN*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, IL 60612

ABSTRACT.—Five ingenane derivatives, 3-*O*-*n*-(deca-2,4,6-trienoyl)-16-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**1**), 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,16,20-*O*-triacetyl-16-hydroxyingenol (**2**), 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16,20-*O*-diacetyl-16-hydroxyingenol (**3**), 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-*O*-acetylingenol (**4**), and 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-acetyl-20-deoxy-16-hydroxyingenol (**5**) were isolated with a new procedure that uses droplet counter-current chromatography, from a dermatitis-producing fraction of the latex of *Euphorbia hermentiana* Lem. The structures of the new compounds **2**, **3**, and **5** were established by the interpretation of their spectroscopic data and those of their hydrolytic and acetylated derivatives.

Euphorbia hermentiana Lem. is a succulent sold as an ornamental plant in the United States. In previous work we have shown that minute quantities of the latex of this species produced irritant follicular dermatitis in open patch tests on human subjects, with the even more severe symptoms of bullae and vesiculation generated when tested in closed patch tests (1). As a continuation of our interest in the skin-irritant principles of commercially available euphorbiaceous species that serve as potential health hazards for humans when they come in contact with the skin or mucous membranes (2-5), we have identified and tested several diterpene acetates present in the hydrolyzed, acetylated latex of *E. hermentiana* (6,7).

In the present contribution we wish to report the identification and characterization of five ingenane derivatives present in an acetone extract of the latex of *E. hermentiana*, which comprise the 16-hydroxyingenol esters, **1-3**, the ingenol ester, **4**, and the 20-deoxy-16-hydroxyingenol ester, **5**. Successful resolution of the *E. hermentiana* latex constituents **1-5** from several esters of the macrocyclic diterpene, ingol, whose characterization will be reported separately, was achieved by means of a combination of droplet counter-current chromatography[3] and preparative tlc. Assignment of the positions of ester substitution in compounds **1-5** was permitted in the present work with the use of stepwise hydrolysis experiments.

Prior to the development of our interest in the constituents of *E. hermentiana*, only one report had been published on the phytochemistry of this species, in which it was found that the tetracyclic triterpenes euphol and euphorbol occur in the latex in a ratio of about 2:1 (8).

RESULTS AND DISCUSSION

The most polar diterpene constituent (**1**) of the skin-irritant acetone extract of *E. hermentiana* latex exhibited spectroscopic properties (uv, ir, pmr, ms) consistent with being a diester of 16-hydroxyingenol (5,9-12), with the acyl substituents being 2,4,6-decatrienoic acid and (*Z*)-2-methyl-2-butenoic (angelic) acid (5,9,12-14). The known

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[1]A contribution from the Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago.

[2]This work was presented, in part, at the 23rd Annual Meeting of the American Society of Pharmacognosy, University of Pittsburgh, Pittsburgh, PA, August 1-5, 1982.

[3]A patent application (U.S. #435070) has been filed by A.D.K. and G.T.M., describing the use of droplet counter-current chromatography for the purification of phorbol esters from croton oil.

compound 16-hydroxyingenol-3,5,16,20-tetraacetate (**8**) was produced on hydrolysis, acetylation, and work-up of **1**. Analysis of the spectroscopic data obtained for **1** indicated a close similarity to those reported for the compound 3-*O*-*n*-(deca-2,4,6-trienoyl)-16-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (Euphorbia factor I_5), which was obtained from the latex of *Euphorbia ingens* E. Mey by the Hecker group, with ester group relative substitution proved by the preparation of a 5,20-acetonide derivative (9,12). In the present work, however, confirmation of the positions of ester substitution in **1** was obtained after mild hydrolysis of this isolate with methanolic potassium hydroxide, which resulted in the formation of the monoester, 16-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**6**). Another product of this reaction from **1** was 16-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-*O*-*n*-(deca-2,4,6-trienoyl)-16-hydroxyingenol (**7**), which was identified on the basis of the appearance, in its pmr spectrum, of two downfield AB quartet resonances typical of a C-16-, C-20-diesterified 16-hydroxyingenol derivative (5). Thus, treatment of the known compound **1** with methanolic KOH led to two products that both retained the ester substituent of **1** at C-16, but in one of which (compound **6**) the C-3 ester group was lost altogether, and in the other (compound **7**) it was translocated to C-20. Attempts to determine the stereochemistry of the double bonds in the 3-acyl group in **1** were hampered by the instability of this isolate.

Compound **2**, the most abundant of the ingenane derivatives obtained in this work, was determined to be a 16-hydroxyingenol ester by comparing its spectroscopic parameters to those of **1**. The molecular formula of **2** was demonstrated to be $C_{31}H_{40}O_{10}$ by hrm spectrometry, and other pmr and mass spectral data indicated that, of the four ester substituents present in this molecule, three were acetate units and one was a (*Z*)-2-methyl-2-butenoate unit. When the pmr spectrum of **2** was compared with those of other ingenol derivatives (5,9,12,15), the observation of resonances at δ 5.05 (sharp singlet), 5.41 (broad singlet), 4.08, 4.24 (AB quartet) and 4.15, 4.61 (AB quartet) ppm, enabled the positions of ester substitution in **2** to be assigned at C-3, C-5, C-16, and C-20, respectively. Work-up of three products obtained by selective hydrolysis of **2** with 0.1 M methanolic KOH resulted in their characterization as 16-*O*-acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**9**), 20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**10**), and 16-*O*-acetyl-16-hydroxyingenol (**11**). However, when compound **9** was acetylated and the resultant tetraester **12** purified and compared with **2** by thin-layer chromatography, **12** was discovered to be a more polar compound than the natural product **2**, thus suggesting that ester group translocation from C-3 to C-20 had occurred during the base hydrolysis of **2** to **9**.

Among the ingenol esters, treatment at various pH's is known to lead to acyl migrations of substituents attached at C-3 to positions C-5 and C-20 (16). The reaction of the commercially available ingenol monoester, 3-*O*-tetradecanoylingenol (**22**), was therefore tested with weak KOH in methanol, in order to rationalize the effect of such treatment on the ingenol ester constituents of *E. hermentiana* latex. It was found that after treatment of **22** with 0.02 M KOH in methanol for 2 min, no starting compound was evident, and that the less polar compound 20-*O*-tetradecanoylingenol (**23**) was the principal reaction product. Therefore, it was suspected that the immediate C-3 to C-20 translocation had occurred on the treatment of the natural product **2** with KOH in methanol.

In contrast, hydrolysis of compound **2** using a strongly acidic cationic exchange resin (17) led to the retention of the C-3 (*Z*)-2-methyl-2-butenoate ester group, but to the selective loss of the C-16 acetate function with the generation of 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,20-*O*-diacetyl-16-hydroxyingenol (**13**). 3-*O*-Tetradecanoylingenol (**22**) remained unaffected by similar treatment.

All of the above evidence pointed to the fact that, in **2**, the (*Z*)-2-methyl-2-butenoate group occurred at C-3, and the three acetate groups were affixed to C-5, C-16, and C-20. Further confirmation of this was obtained by the appearance, in the pmr spectrum of **2** of the C-3 methine proton at a position further downfield (δ 5.05) than that of **12** (δ 4.96), an observation in accord with the presence of a more electronegative C-3 ester substituent in the former compound. Thus, **2** was elucidated as 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,16,20-*O*-triacetyl-16-hydroxyingenol, and is the first example of a naturally occurring 16-hydroxyingenol tetraester to have been structurally characterized to date.

Analysis of the pmr and mass spectra of **3** suggested that it was a 16-hydroxyingenol triester, containing two acetate moieties and one (*Z*)-2-methyl-2-butenoate moiety, with esterification occurring at C-3, C-16 and C-20. Because compound **2** was exclusively produced on acetylation of **3**, it may be inferred that the latter is 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16,20-*O*-diacetyl-16-hydroxyingenol.

The ingenol diester, **4**, yielded ingenol-3,5,20-triacetate (**17**) on saponification and acetylation, and 20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**15**) on mild base hydrolysis. On acetylation, **15** produced 3,5-*O*-diacetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**16**), a compound found to be virtually inseparable by tlc from an acetylate of **4**, 3-*O*[(*Z*)-2-methyl-2-butenoyl]-5,20-*O*-diacetylingenol (**14**). However, analogous to the situation already described for compounds **2** and **12**, the C-3 methine proton pmr resonance of **14** occurred downfield (δ 5.07) of that of **16** (δ 4.99). This observation, coupled with the demonstration of the facile C-3 ester translocation with base in the model compound **22**, strongly suggested that the structure of **4** was 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-*O*-acetylingenol,[4] a compound reported recently as a new natural product from the latex of *Euphorbia kamerunica* Pax (18).

The final ingenane derivative to be obtained in these isolation experiments, **5**, was typified by an additional vinyl methyl signal in its pmr spectrum, when compared with pmr data recorded for compounds **1-4**. Compound **5**, on complete hydrolysis and acetylation, afforded the resinous derivative 20-deoxy-16-hydroxyingenol-3,5,16-triacetate (**21**), a compound obtained previously in our work on *E. hermentiana* latex (6,7). It may be noted that a crystalline derivative of **5**, compound **18**, was obtained on direct acetylation, without prior hydrolysis. The diester **5** was assigned one acetate and one (*Z*)-2-methyl-2-butenoate substituent on analysis of its pmr and mass spectra. In a similar manner to compounds **1, 2** and **4**, ester group translocation occurred during selective hydrolysis of **5** with methanolic KOH. Two products were characterized, namely, 5-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-acetyl-20-deoxy-16-hydroxyingenol (**19**) and 5-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-deoxy-16-hydroxyingenol (**20**). Compound **19** was distinguishable from **5** by pmr observations of a diamagnetic shift of the sharp singlet of the C-3 methine proton (δ 5.47 in **5** vs. δ 3.47 in **19**) and the concomitant paramagnetic shift of the broad singlet of the C-5 proton (δ 5.24 in **19** vs. δ 3.69 in **5**). Therefore, **5** was assigned the structure 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-acetyl-20-deoxy-16-hydroxyingenol, and is the first naturally occurring ester of this parent diterpene alcohol to be isolated to date.

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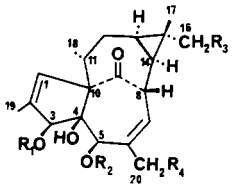
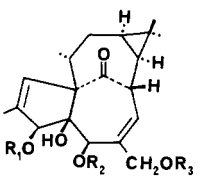
[4]Compound **4** was identical to a constituent of *E. canariensis* L. latex that was recently reported by us as having the structure 3-*O*-acetyl-20-*O*[(*Z*)-2-methyl-2-butenoyl]ingenol (5). In view of the facile C-3 to C-20 ester substituent shift demonstrated to occur with weak KOH in methanol in many of the ingenol-3-esters we have studied, such an assignment may now be regarded as erroneous. With this in mind, it is likely that another *E. canariensis* latex constituent, identified by us as 3-*O*-acetyl-16-*O*-benzoyl-20-*O*[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (5), as well as 3-*O*-propionyl-20-*O*-(*S*)-(2′ methyl-butyryl-ingenol, identified as a *E. cotinifolia* L. constituent by Hirota and co-workers (15), are correctly assigned with a reversal of their C-3 and C-20 ester substituents, inasmuch as both compounds were subjected to mild base hydrolysis in the course of their investigation.

None of the isolates **1-5** were biologically tested in the course of the present study. However, **1** is known to be an extremely potent skin-irritant for mouse skin, as well as being a weak tumor-promoting agent (12). Similarly, we have found that **4** produces severe inflammation when applied to mouse ears at a dose of 10 μg/5 μl (19). Therefore, it is likely that **1** and **4** are mainly responsible for the inflammatory signs and symptoms produced in humans from contact with the skin by *E. hermentiana* latex. In addition, since we have found that the C-20 acetylated ingenol triester (**17**) was a weak skin-irritant when used in closed patch tests on human skin (6), it is possible that compounds **2** and **3** also contribute to the overall dermatological toxicity of *E. hermentiana* latex for humans. However, the C-20 deoxygenated compound **5** is probably inactive in this respect, inasmuch as our previous experiments have shown that compound **21** was devoid of skin-irritant activity in open and closed patch tests on human skin at a relatively high applied dose of 50 μg/5 μl.





1 $R_1 = C_3H_7(CH{=}CH)_3CO$; $R_2 = H$; R_3 =  ; $R_4 = OH$

2 R_1 =  ; $R_2 = CH_3CO$; $R_3 = R_4 = CH_3COO$

3 R_1 =  ; $R_2 = H$; $R_3 = R_4 = CH_3COO$

5 R_1 =  ; $R_2 = H$; $R_3 = CH_3COO$; $R_4 = H$

6 $R_1 = R_2 = H$; R_3 = ; $R_4 = OH$

7 $R_1 = R_2 = H$; R_3 =  ; $R_4 = C_3H_7(CH{=}CH)_3COO$

8 $R_1 = R_2 = CH_3CO$; $R_3 = R_4 = CH_3COO$

9 $R_1 = R_2 = H$; $R_3 = CH_3COO$; R_4 = 

10 $R_1 = R_2 = H$; $R_3 = OH$; R_4 = 

11 $R_1 = R_2 = H$; $R_3 = CH_3COO$; $R_4 = OH$

12 $R_1 = R_2 = CH_3CO$; $R_3 = CH_3COO$; R_4 = 

13 R_1 =  ; $R_2 = CH_3CO$; $R_3 = OH$; $R_4 = CH_3COO$

14 R_1 =  ; $R_2 = CH_3CO$; $R_3 = CH_3COO$; $R_4 = H$

19 $R_1 = H$; R_2 =  ; $R_3 = CH_3COO$; $R_4 = H$

20 $R_1 = H$; R_2 =  ; $R_3 = OH$; $R_4 = H$

21 $R_1 = R_2 = CH_3CO$; $R_3 = CH_3COO$; $R_4 = H$

4 R_1 =  ; $R_2 = H$; $R_3 = CH_3CO$

14 R_1 =  ; $R_2 = R_3 = CH_3CO$

15 $R_1 = R_2 = H$; R_3 = 

16 $R_1 = R_2 = CH_3CO$; R_3 =

17 $R_1 = R_2 = R_3 = CH_3CO$

22 $R_1 = C_{13}H_{27}CO$; $R_2 = R_3 = H$

23 $R_1 = R_2 = H$; $R_3 = C_{13}H_{27}CO$

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured with a Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Uv spectra were obtained on a Beckman model DB-G grating spectrophotometer, using methanol as solvent; ir spectra were obtained either in chloroform using a Beckman model 18-A spectrophotometer, with polystyrene calibration at 1601 cm^{-1}, or on a Nicolet MX-1 FT-IR spectrophotometer, using an AgCl cell. Pmr spectra were recorded in deuterochloroform, using TMS as internal standard, on either a Varian T-60A instrument, with a Nicolet TT-7 Fourier Transform attachment (60 MHz), or on a Nicolet NT-360 instrument (360 MHz). Low-resolution mass spectra were obtained on a Varian MAT 112S instrument (*ca.* 20 eV), and high-resolution mass spectra were obtained by peak-matching on an AEI MS-902 instrument (70 eV). Droplet counter-current chromatography (DCCC) was performed at room temperature on a Model A instrument (Tokyo Rikakikai, Tokyo, Japan); preparative tlc was conducted on silica gel GHLF (Analtech Inc., Newark, Delaware), with 250 μm thick layers, using three solvent systems, namely, hexane-benzene-diethyl ether-ethyl acetate (2:2:1:1, solvent 1), cyclohexane-diethyl ether-ethyl acetate (1:1:1, solvent 2) and chloroform-diethyl ether (19:1, solvent 3). The tlc plates were visualized using 70% w/v sulfuric acid with the application of heat.

PLANT MATERIAL.—Specimens of *Euphorbia hermentiana* Lem. were purchased from plant stores in the Chicago area, and identified by Dr. D.D. Soejarto, as described previously (1,6). Latex aliquots (*ca.* 10 ml) were collected from the specimens at twice-weekly intervals over a period of four months and were dried at 40° under reduced pressure.

EXTRACTION AND FRACTIONATION.—Dried latex (27 g) was exhaustively extracted with acetone, and the residue (6.5 g) partitioned between hexane (60 ml) and methanol-water (17:3, 2 x 30 ml). The combined polar layers were then adjusted to a 1:1 v/v methanol-water ratio by the addition of 42 ml water, and were then extracted with 3 x 100 ml methylene chloride. On drying, the residue from the less polar solvent (0.7 g) was fractionated by DCCC using a saturated mixture of hexane-ether-1-propanol-ethanol-water (7:16:6:10:8) as developing solvent, with the upper phase employed as mobile phase. The solute was dissolved in 5 ml mobile phase, and, on the addition of 5 ml of stationary phase, was introduced into a 10-ml sample chamber. Ascending development was employed at a pressure of 2-4 kg/cm^2, and fractions (120 drops each) were collected into an automatic fraction collector.

Fractions 38-50 from the DCCC separation were purified by sequential preparative tlc in solvent 1 (Rf 0.09) and solvent 2 (Rf 0.30), to afford 13.2 mg of compound **1**. DCCC fractions 51-109 exhibited three major zones when solvent 1 was used for development. The most polar zone was purified by preparative tlc in solvent 3 to yield 17.6 mg of compound **2** (Rf 0.21), 1.8 mg of compound **4** (Rf 0.17) and 17.8 mg of compound **5** (Rf 0.12). When fractions 110-174 from the DCCC apparatus were subjected to preparative tlc with solvent 1 as developer, 1.5 mg of compound **3** (Rf 0.16) were obtained.

This isolation procedure was repeated on 56 g of dried *E. hermentiana* latex to produce, respectively, a further 19.9 mg, 32.8 mg, 6.3 mg, 3.4 mg, and 26.4 mg of compounds **1-5**.

CHARACTERIZATION OF COMPOUND **1**.—The resinous 3-*O*-*n*-(deca-2,4,6-trienoyl)-16-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**1**, 33.1 mg, 0.04% w/w) exhibited the following data: $[\alpha]^{25}D$ +17.0° (c 0.17, $CHCl_3$); uv, λ max 305 (log ε 4.28) and 214 nm (4.26); ir, ν max ($CHCl_3$) 3300-3500, 2950, 2900, 1700, 1600, 1450, 1370, 1295, and 1260 cm^{-1}; pmr, (60 MHz) δ 0.98 (3H, d, J=6.6 Hz, 18-CH_3), 1.15 (3H, s, 17-CH_3), 1.80 (3H, br s, 19-CH_3), 1.93, 2.06 (3H each, m, 4′-CH_3, 5′-CH_3), 3.55 (1H, m, exchangeable with D_2O, -OH), 4.07 (1H, s, 5-H), 4.13 (2H, s, 20-H_2), 4.13, 4.48 (2H, AB_q, J_{AB}=12.1 Hz, 16-H_2), 5.63 (1H, s, 3-H), and 5.76-7.77 (9H, m, 1-H, 7-H, 3′-H and olefinic H); ms, *m/z* 594 (M^+, 2%), 472 (1), 429 (1), 428 (1), 411 (1), 354 (3), 328 (7), 311 (10), 310 (9), 292 (8), 282 (5), 281 (8), 252 (7), 166 (4), 150 (12), 123 (25), 122 (14), 100 (34), and 83 (100). These data are closely comparable with spectroscopic data published for Euphorbia factor I_5 (**1**) (9,12), a constituent of *E. ingens* E. Mey latex.

Compound **1** (14 mg) was hydrolyzed with 0.02 M KOH in dry methanol for 2 min at room temperature. Two major products were observed on tlc analysis, with the more polar compound purified by preparative tlc in solvent 2 (Rf 0.06) to give the resinous 16-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**6**, 2 mg), which exhibited the following data: $[\alpha]^{25}D$ −21.6° (c 0.042, $CHCl_3$); uv λ max 268 (log ε 3.60) and 208 nm (4.06); ir, ν max (AgCl) 3454, 3404, 2950, 2925, 1763, 1605, 1510, 1430, 1363, and 1275 cm^{-1}; pmr, (60 MHz) δ 0.99 (3H, d, J=8.7 Hz, 18-CH_3), 1.12 (3H, s, 17-CH_3), 1.85 (9H, m, 19-CH_3, 4′-CH_3, 5′-CH_3), 2.53-3.02, 3.71 (2H, m, exchangeable with D_2O, 2 x -OH), 3.82 (1H, br s, 5-H), 4.15 (2H, s, 20-H_2), 4.38 (1H, s, 3-H), 4.27, 4.52 (2H, AB_q, J_{AB}=13.4 Hz, 16-H_2), 5.92 (1H, s, 1-H), 6.05 (1H, m, 7-H), and 6.15 ppm (1H, m, 3′-H); ms, *m/z* 446 (M^+, 1%), 428 (1), 410 (1), 392 (1), 348 (2), 330 (8), 312(19), 294 (15), 284 (18), 283 (16), 269 (22), 251 (22), 241 (31), 179 (41), 162 (54), 151 (93), 135 (89), 123 (81), 122 (100), and 83 (64).

Purification of the less polar compound from this hydrolysis procedure, using preparative tlc in solvent 2 (Rf 0.43), afforded 16-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-*O*-*n*-(deca-2,4,6-trienoyl)-16-hydroxyingenol (**7**, 2 mg), a less polar compound than **1**, which exhibited the following data: $[\alpha]^{25}D$ −7.7° (c 0.042, $CHCl_3$); uv, λ max 305 (log ε 4.25) and 209 nm (4.20); ir, ν max (AgCl) 3454, 3376, 2956, 2937, 1765, 1756, 1603, 1514, and 1273 cm^{-1}; pmr, (60 MHz) δ 0.98 (3H, d, J=6.7 Hz, 18-CH_3), 1.17 (3H, s, 17-CH_3), 1.87-1.98 (9H, m, 19-CH_3, 4′-CH_3, 5′-CH_3), 2.22-2.65 (1H, m, exchangeable with D_2O, -OH), 3.72 (1H, br s, 5-H), 4.15 (1H, m, 8-H), 4.25, 4.50 (2H, AB_q, J_{AB}=10.5 Hz, 16-H_2), 4.39 (1H, s, 3-H), 4.57, 4.81 (2H, AB_q, J_{AB}=11.5 Hz, 20-H_2), and 5.72-7.77 ppm (9H, m, 1-H, 7-H, 3′-H and olefinic H); ms, *m/z* 594 (M^+, 6%), 494 (4), 476 (2), 429 (12), 411 (6), 330 (12), 329 (13), 313 (17), 312 (17), 311 (31), 293 (11), 283 (14), 264 (26), 223 (14), 189 (16), 166 (23), 150 (26), 100 (13), and 83 (100).

Compound **1** (2 mg) was hydrolyzed in 0.5 M KOH in dry methanol for 30 min at room temperature, and the product was acetylated in pyridine-acetic anhydride (4:1, 0.5 ml) for 1 h at 100°. Extraction into $CHCl_3$ and preparative tlc in solvent 3 (Rf 0.11) afforded the resinous 16-hydroxyingenol-3,5,16,20-tetraacetate (**8**, 1 mg). Identity of **8** was confirmed by comparison (ms, co-tlc) with an authentic sample of this compound obtained from the latex of *E. canariensis* L. (5).

CHARACTERIZATION OF COMPOUND **2**.—Crystalline 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,16,20-*O*-

triacetyl-16-hydroxyingenol (**2**, 50.4 mg, 0.061% w/w) was found to exhibit the following data: mp, 127-128°; $[\alpha]^{25}D$ +7.5° (c 0.13, $CHCl_3$); uv, λ max 215 nm (log ε 4.37); ir, ν max ($CHCl_3$) 3525, 2950, 2900, 1715, 1440, 1370, 1355, and 1235 cm^{-1}; pmr, (60 MHz) δ 0.99 (3H, d, J=7.1 Hz, 18-CH_3), 1.13 (3H, s, 17-CH_3), 1.76 (3H, br s, 19-CH_3), 1.91, 2.00 (3H each, m, 4′-CH_3, 5′-CH_3), 2.00, 2.06, 2.26 (3H each, s, 3 x -$OCOCH_3$), 3.34 (1H, m, exchangeable with D_2O, 4-OH), 4.08, 4.28 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 4.15, 4.61 (2H, AB_q, J_{AB}=12.4 Hz, 20-H_2), 5.05 (1H, s, 3-H), 5.41 (1H, br s, 5-H), 6.06 (1H, s, 1-H), 6.12 (1H, m, 7-H), and 6.20 ppm (1H, m, 3′-H); ms, *m/z* 572 (M^+, 2%), 513 (3), 472 (4), 454 (1), 412 (19), 394 (3), 384 (20), 370 (13), 352 (19), 328 (4), 310 (21), 293 (12), 292 (20), 264 (15), 151 (16), 135 (16), 122 (20), 121 (27), 95 (13), and 83 (100); mass measurement: found 572.2620, calcd for $C_{31}H_{40}O_{10}$, 572.2620.

Compound **2** (10 mg) was partially hydrolyzed with 0.1 M KOH in dry methanol for 20 min at room temperature. Three products were observed as a result of this hydrolysis, which were purified by preparative tlc in solvent 2. The major reaction product, and least polar compound (Rf 0.24), 16-*O*-acetyl-20-*O*-[(*Z*)-methyl-2-butenoyl]-16-hydroxyingenol (**9**, 3 mg), was found to exhibit the following data: resin: $[\alpha]^{25}D$ −32.2° (c, 0.039, $CHCl_3$); uv, λ max 215 nm (log ε 4.12); ir, ν max (AgCl) 3410, 2935, 1720, 1380, and 1230 cm^{-1}; pmr, (60 MHz) δ 0.96 (3H, d, J=6.3 Hz, 18-CH_3), 1.14 (3H, s, 17-CH_3), 1.85 (3H, br s, 19-CH_3), 1.88, 2.05 (3H each, m, 4′-CH_3, 5′-CH_3), 2.16 (3H, s, -$OCOCH_3$), 3.68 (1H, br s, 5-H), 4.15 (1H, m, 8-H), 4.15, 4.36 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 4.40 (1H, s, 3-H), 4.59, 4.81 (2H, AB_q, J_{AB}=12.4 Hz, 20-H_2), 5.89 (1H, s, 1-H), 6.06 (1H, m, 7-H), and 6.10 ppm (1H, m, 3′-H); ms, *m/z* 488 (M^+, 1%), 470 (1), 452 (1), 428 (2), 410 (3), 388 (5), 370 (9), 352 (7), 328 (15), 311 (11), 310 (25), 292 (13), 281 (16), 189 (20), 160 (52), 151 (65), 135 (37), 123 (40), 122 (43), 121 (32), 100 (16), 95 (32), and 83 (100).

The product of intermediate polarity (Rf 0.05), 20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**10**, 2 mg), was found to exhibit the following data: resin; $[\alpha]^{25}D$ −9.6° (c 0.10, $CHCl_3$); uv, λ max 210 nm (log ε 4.13); ir, ν max (AgCl) 3370-3470, 2935, 1720, 1460, and 1250 cm^{-1}; pmr, (60 MHz) δ 0.98 (3H, d, J=7.5 Hz, 18-CH_3), 1.17 (3H, s, 17-CH_3), 1.85 (3H, br s, 19-CH_3), 1.88 (6H, m, 4′-CH_3, 5′-CH_3), 3.72 (1H, br s, 5-H), 3.81 (2H, s, 16-H_2), 3.88 (1H, m, exchangeable with D_2O, -OH), 4.21 (1H, m, 8-H), 4.41 (1H, s, 3-H), 4.59, 4.81 (2H, AB_q, J_{AB}=12.4 Hz, 20-H_2), 5.94 (1H, d, J=2.3 Hz, 1-H), 6.06 (1H, m, 7-H), and 6.16 ppm (1H, mm, 3′-H); ms, *m/z* M^+ missing, 346 (M^+-100, 5%), 328 (5), 311 (4), 310 (3), 292 (5), 282 (28), 249 (15), 189 (12), 123 (12), 122 (9), 100 (10), 95 (18), and 83 (100).

The product of greatest polarity from this reaction (Rf 0.02), 16-*O*-acetyl-16-hydroxyingenol (**11**, 1.5 mg), gave the following data: resin: $[\alpha]^{25}D$ −15.8° (c 0.042, $CHCl_3$), uv, λ max 209 nm (log ε 4.12); ir, ν max (AgCl) 3390-3460, 2924, 1723, 1713, 1380, and 1240 cm^{-1}; pmr, (60 MHz) δ 0.97 (3H, d, J=7.3 Hz, 18-CH_3), 1.15 (3H, s, 17-CH_3), 1.84 (3H, d, J=1.0 Hz, 19-CH_3), 2.08 (3H, s, -$OCOCH_3$), 2.63-3.07 (1H, m, exchangeable with D_2O, -OH), 3.68 (1H, m, exchangeable with D_2O, -OH), 3.81 (1H, br s, 5-H), 4.12 (2H, s, 20-H_2), 4.13, 4.39 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 4.39 (1H, s, 3-H), 5.88 (1H, d, J=2.6 Hz, 1-H), and 6.01 ppm (1H, m, 7-H); ms, *m/z* 406 (M^+, 6%), 346 (8), 328 (13), 310 (13), 292 (10), 282 (15), 264 (20), 189 (36), 177 (31), 151 (59), 123 (94), 122 (65), and 83 (100).

Compound **9** (16-*O*-acetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol, 1.5 mg) was acetylated in pyridine-acetic anhydride (4:1, 0.5 ml) for 1 h at 100°. Extraction into chloroform and preparative tlc in solvent 1 (Rf 0.25) afforded 3,5,16-*O*-triacetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-hydroxyingenol (**12**, 1.3 mg), which exhibited the following data: resin; $[\alpha]^{25}D$ +5.0° (c 0.10, $CHCl_3$); uv, λ max 212 nm (log ε 4.36); ir, ν max (AgCl) 3454, 2961, 2925, 1737, 1730, 1697, 1460, 1370, 1235, 1231, 1152, and 1025 cm^{-1}; pmr, (60 MHz) δ 1.00 (3H, d, J=8.2 Hz, 18-CH_3), 1.13 (3H, s, 17-CH_3), 1.75 (3H, br s, 19-CH_3), 1.86, 2.06 (3H each, m, 4′-CH_3, 5′-CH_3), 2.06, 2.11, 2.22 (3H each, s, 3 x -$OCOCH_3$), 3.24 (1H, br s, exchangeable with D_2O, 4-OH), 4.09, 4.30 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 4.24, 4.64 (2H, AB_q, J_{AB}=12.4 Hz, 20-H_2), 4.96 (1H, s, 3-H), 5.38 (1H, br s, 5-H), 6.06 (1H, s, 1-H), 6.13 (1H, m, 7-H), and 6.20 ppm (1H, m, 3′-H); ms, *m/z*, M^+ missing, 512 (M^+-60, 1%), 452 (3), 396 (2), 370 (5), 352 (8), 310 (9), 292 (10), 264 (7), 249 (5), 236 (14), 193 (6), 177 (4), 151 (8), 135 (7), 122 (6), 121 (7), and 83 (100).

Compound **12** was separable from compound **2** by analytical tlc in solvents 1 (Rf 0.25 vs. 0.31), 2 (Rf 0.50 vs. 0.53), and 3 (Rf 0.17 vs. 0.21), respectively.

Compound **2** (6 mg) was also partially hydrolyzed with Dowex 50W-X2[5] (60 mg, 100-200 mesh, H^+ form) ion-exchange resin (17), suspended in methanol-water (10:1, 1 ml). The major reaction product, 3-*O*-[(*Z*]-2-methyl-2-butenoyl]-5,20-*O*-diacetyl-16-hydroxyingenol (**13**, 1.5 mg), which was purified by preparative tlc in solvent 2 (Rf 0.23), was found to exhibit the following data: resin; uv, λ max 215 nm (log ε 4.32); ir, ν max (AgCl) 3300-3500, 2920, 1735, 1700, 1685, 1460, 1385, 1248, 1155, and 1025 cm^{-1}; pmr, (60 MHz) δ 1.00 (3H, d, J=7.2 Hz, 18-CH_3), 1.17 (3H, s, 17-CH_3), 1.78 (3H, br s,

[5]Bio-Rad Laboratories, Richmond, CA.

19-CH_3), 1.91, 2.00 (3H each, m, 4′-CH_3, 5′CH_3), 2.00, 2.25 (3H each, 2 x -OCOCH_3), 3.38 (1H, s, exchangeable with D_2O, -OH), 3.76 (2H, s, 16-H_2), 4.01, 4.79 (2H, AB_q, J_{AB}= 12.0 Hz, 20-H_2), 5.07 (1H, s, 3-H), 5.43 (1H, br s, 5-H), 6.08 (1H, s, 1-H), 6.17 (1H, m, 7-H), and 6.22 ppm (1H, m, 3′-H); ms, *m/z* M^+ missing, 430 (M^+-100, 2%), 412 (3), 370 (6), 328 (9), 310 (11), 292 (5), 282 (8), 269 (10), 249 (7), 188 (11), 151 (11), 135 (13), 122 (14), and 83 (100). Compound **2** was regenerated on the acetylation of **13** under the conditions described for **9**.

When compound **2** (2 mg) was hydrolyzed with 0.5 M KOH in dry methanol for 30 min, acetylated, and worked up as described for **1**, 16-hydroxyingenol-3,5,16,20-tetraacetate (**8**, 0.5 mg) was obtained and was found to compare favorably (ms, co-tlc) with an authentic sample of this compound (5).

CHARACTERIZATION OF COMPOUND **3**.—Resinous 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16,20-*O*-diacetyl-16-hydroxyingenol (**3**, 7.8 mg, 0.0094% w/w) exhibited the following data: $[\alpha]^{25}D$ +15.0° (c 0.16, $CHCl_3$); uv, λ max 217 nm (log ε 4.24); ir, ν max ($CHCl_3$) 3480, 2950, 2910, 1715, 1440, 1370, and 1220 cm^{-1}; pmr, (60 MHz) δ 0.97 (3H, d, J=7.0 Hz, 18-CH_3), 1.13 (3H, s, 17-CH_3), 1.80 (3H, d, J=1.3 Hz, 19-CH_3), 1.94, 2.06 (3H each, m, 4′-CH_3, 5′-CH_3), 2.06 (6H, s, 2 x -OCOCH_3), 3.88 (1H, br s, 5-H), 4.19 (1H, m, 8-H), 4.05, 4.35 (2H, AB_q, J_{AB}= 12.6 Hz, 16-H_2), 4.49, 4.74 (2H, AB_q, J_{AB}= 13.4 Hz, 20-H_2), 5.58 (1H, s, 3-H), 6.03 (1H, d, J=1.5 Hz, 1-H), 6.10 (1H, m, 7-H), and 6.16 ppm (1H, m, 3′-H); ms, *m/z* 530 (M^+, 0.3%), 471 (1), 430 (1), 412 (1), 371 (4), 370 (10), 352 (5), 328 (3), 310 (9), 292 (6), 282 (8), 187 (12), 160 (16), 153 (26), 151 (25), 135 (15), 122 (32), 121 (27), and 83 (100).

Compound **3** (1.5 mg) was acetylated and extracted into chloroform as described for compound **1**, and the product (1.5 mg), when purified by preparative tlc in solvent 1 (Rf 0.31), was shown to be identical to **2** by direct (mp, $[\alpha]_D$, uv, ir, pmr, ms, co-tlc) comparison.

CHARACTERIZATION OF COMPOUND **4**.—Resinous 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-20-*O*-acetylingenol (**4**, 5.2 mg, 0.0063% w/w) exhibited the following properties: $[\alpha]^{25}D$ +24.9° (c 0.13, $CHCl_3$); uv, λ max 218 nm (log ε 4.35); ir, ν max ($CHCl_3$) 3520, 2920, 1715, 1440, 1370, and 1220 cm^{-1}; pmr (60 MHz) δ 0.97 (3H, d, J=7.0 Hz, 18-CH_3), 1.06, 1.08 (3H each, s, 16-CH_3, 17-CH_3), 1.79 (3H, br s, 19-CH_3), 1.94, 2.05 (3H each, m, 4′-CH_3, 5′-CH_3), 2.05 (3H, s, -OCOCH_3), 2.84-3.35 (1H, m, exchangeable with D_2O, -OH), 3.89 (1H, br s, 5-H), 4.09 (1H, m, 8-H), 4.52, 4.77 (2H, AB_q, J_{AB}= 12.0 Hz, 20-H_2), 5.58 (1H, s, 3-H), 6.05 (1H, d, J=1.4 Hz, 1-H), 6.12 (1H, m, 7-H), and 6.20 ppm (1H, m, 3′-H); ms, *m/z* 472 (M^+, 0.5%), 454 (2), 412 (1), 394 (1), 372 (5), 354 (4), 330 (3), 329 (3), 312 (31), 294 (22), 284 (21), 251 (13), 221 (16), 188 (23), 153 (34), 151 (35), 135 (39), 122 (99), 121 (64), and 83 (100).

Compound **4** (1.5 mg) was acetylated and worked up as described for **9**, with 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,20-*O*-diacetylingenol (**14**, 1.4 mg) being obtained. This acetylate exhibited the following data: resin; $[\alpha]^{25}D$+ 7.7° (c 0.17, $CHCl_3$); uv, λ max 212 nm (log ε 4.08); ir, ν max ($CHCl_3$) 3520, 2950, 2900, 1730, 1715, 1460, 1370, 1360, and 1220 cm^{-1}; pmr, (60 MHz) δ 0.99 (3H, d, J=8.0 Hz, 18-CH_3), 1.06, 1.08 (3H each, s, 16-CH_3, 17-CH_3), 1.77 (3H, d, J=1.1 Hz, 19-CH_3), 1.91, 2.00 (3H each, m, 4′-CH_3, 5′-CH_3), 2.16, 2.25 (3H each, s, 2 x -OCOCH_3), 3.28 (1H, s, exchangeable with D_2O, 4-OH), 4.18 (1H, m, 8-H), 4.04, 4.76 (2H, AB_q, J_{AB}= 12.0 Hz, 20-H_2), 5.07 (1H, s, 3-H), 5.42 (1H, br s, 5-H), 6.08 (1H, d, J=1.5 Hz, 1-H), 6.18 (1H, m, 7-H), and 6.20 ppm (1H, m, 3′-H); ms, *m/z* M^+ missing, 414 (M^+-100, 4%), 372 (3), 354 (7), 312 (16), 294 (13), 279 (37), 122 (16), 112 (16), and 83 (100).

Compound **4** (2.9 mg) was hydrolyzed with 0.1 M KOH in dry methanol for 10 min at room temperature. The less polar of two hydrolyzed products, 20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**15**, 1.5 mg), on work-up and purification by preparative tlc in solvent 2 (Rf 0.38), exhibited the following data: resin; uv, λ max 212 nm (log ε 4.02); ir, ν max ($CHCl_3$) 3440, 3427, 3406, 2957, 2873, 1725, 1712, 1440, and 1380 cm^{-1}; pmr, (60 MHz) δ 0.97 (3H, d, J=7.2 Hz, 18-CH_3), 1.06, 1.11 (3H each, s, 16-CH_3, 17-CH_3), 1.83, 1.85, 1.87 (3H each, m, 19-CH_3, 4′-CH_3, 5′-CH_3), 3.69 (1H, br s, 5-H), 4.10 (1H, m, 8-H), 4.42 (1H, s, 3-H), 4.59, 4.78 (2H, AB_q, J_{AB}= 12.0 Hz, 20-H_2), 5.93 (1H, d, J=1.1 Hz, 1-H), 6.02 (1H, m, 7-H), and 6.11 ppm (1H, m, 3′-H); ms, *m/z* 430 (M^+, 6%), 412 (4), 394 (4), 330 (8), 312 (19), 294 (16), 284 (17), 241 (17), 162 (41), 151 (47), 147 (41), 135 (42), 122 (56), 109 (34), 97 (65), and 83 (100).

Compound **15** (1.1 mg) was acetylated and worked up as described for **9**, and the resultant 3,5-*O*-diacetyl-20-*O*-[(*Z*)-2-methyl-2-butenoyl]ingenol (**16**, 1 mg) was found to exhibit the following data when purified: resin; uv, λ max 212 nm (log ε 4.12); ir, ν max (AgCl) 3532, 2930, 1750, 1730, 1460, 1380, and 1235 cm^{-1}; pmr, (60 MHz) δ 0.98 (3H, d, J=7.2 Hz, 18-CH_3), 1.08 (6H, s, 16-CH_3, 17-CH_3), 1.76 (3H, d, J=1.4 Hz, 19-CH_3), 1.87, 1.98 (3H each, m, 4′-CH_3, 5′-CH_3), 2.12, 2.21 (3H each, s, 2 x -OCOCH_3), 3.24 (1H, m, exchangeable with D_2O, 4-OH), 4.22 (1H, m, 8-H), 4.20, 4.49 (2H, AB_q, J_{AB}= 11.4 Hz, 20-H_2), 4.99 (1H, s, 3-H), 5.40 (1H, br s, 5-H), 6.09 (1H, s, 1-H), 6.13 (1H, m, 7-H),

and 6.20 ppm (1H, m, 3′-H); ms, *m/z* M^+ missing, 454 (M^+-60, 4%), 394 (3), 354 (6), 313 (15), 312 (23), 294 (23), 284 (9), 266 (14), 251 (11), 223 (7), 151 (7), 135 (11), 122 (11), 121 (12), and 83 (100).

Compounds **14** and **16** were not clearly separable by analytical tlc in solvents 1-3.

Complete hydrolysis of **4** (1.0 mg) in 0.5 M KOH in methanol, and acetylation and work up, similar to the procedure described for **1**, resulted in the formation of ingenol-3,5,20-triacetate (**17**, 0.5 mg), which was identified by comparison (mp, ms, co-tlc) with a sample of this compound obtained earlier in our work on *E. hermentiana* latex (6).

CHARACTERIZATION OF COMPOUND **5**.—Resinous 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-acetyl-20-deoxy-16-hydroxyingenol (**5**, 44.2 mg, 0.053% w/w) exhibited the following data: $[\alpha]^{25}D$ +16.9° (c 0.28, $CHCl_3$); uv, λ max 215 nm (log ε 4.19); ir, ν max ($CHCl_3$) 3530, 3480, 2950, 2900, 1710, 1630, 1440, 1370, and 1230 cm^{-1}; pmr, (360 MHz) δ 0.91 (1H, m 14-H), 0.98 (3H, d, J=7.1 Hz, 18-CH_3), 1.12 (3H, s, 17-CH_3), 1.78 (3H, s, 20-CH_3), 1.80 (3H, br s, 19-CH_3), 1.92, 2.01 (3H each, m, 4′-CH_3, 5′-CH_3), 2.07 (3H, s, -OCOCH_3), 2.30 (2H, m, 12-H_2), 3.13, 3.46 (2H, m, exchangeable with D_2O, 2 x -OH), 3.69 (1H, br s, 5-H), 4.13 (1H, m, 8-H), 4.13, 4.28 (2H, AB_q, J_{AB}=11.9 Hz, 16-H_2), 5.47 (1H, s, 3-H), 5.74 (1H, d, J=3.5 Hz, 7-H), 6.05 (1H, d, J=1.4 Hz, 1-H), and 6.18 ppm (1H, m, 3′-H); ms, *m/z* 472 (M^+, 1%), 454 (1), 414 (1), 413 (2), 372 (5), 354 (4), 330 (1), 329 (2), 312 (21), 294 (11), 251 (9), 221 (22), 189 (18), 162 (45), 151 (34), 135 (33), 122 (85), 121 (55), 95 (93), and 83 (100); mass measurement: found 472.2465, calcd for $C_{27}H_{36}O_7$, 472.2459.

Compound **5** (6.3 mg) was acetylated in pyridine-acetic anhydride (2:1, 0.5 ml) for 6 h at room temperature. The acetylated product was extracted into chloroform, and purified by preparative tlc in solvent 1 (Rf 0.50). This isolate, 3-*O*-[(*Z*)-2-methyl-2-butenoyl]-5,16-*O*-diacetyl-20-deoxy-16-hydroxyingenol (**18**, 5.2 mg) was crystallized from acetone, mp, 125-126°; $[\alpha]^{25}D$ +2.9° (c, 0.077, $CHCl_3$); uv, λ max 215 nm (log ε 4.29); ir, ν max ($CHCl_3$) 3520, 2950, 1710, 1430, 1350, and 1200 cm^{-1}; pmr (60 MHz) δ 0.98 (3H, d, J=7.1 Hz, 18-CH_3), 1.12 (3H, s, 17-CH_3, 5′-CH_3), 1.56 (3H, br s, 20-CH_3), 1.76 (3H, br s, 19-CH_3), 1.91, 2.06 (3H each, m, 4′-CH_3), 2.06, 2.30 (3H each, s, 2 x -OCOCH_3), 3.29 (1H, m, exchangeable with D_2O, 4-OH), 4.11, 4.28 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 5.02 (1H, s, 3-H), 5.25 (1H, br s, 5-H), 5.83 (1H, br d, J=4.2 Hz, 7-H), 6.07 (1H, s, 1-H), and 6.12 ppm (1H, m, 3′-H; ms, *m/z* 514 (M^+, 3%), 455 (8), 415 (4), 414 (10), 396 (3), 372 (12), 355 (16), 354 (40), 336 (14), 326 (11), 313 (15), 312 (40), 311 (10), 295 (22), 294 (39), 284 (12), 279 (15), 266 (27), 251 (19), 189 (18), 162 (23), 161 (23), 151 (27), 135 (42), 122 (49), 121 (58), 95 (37), and 83 (100).

Compound **5** (12.1 mg) was partially hydrolyzed with 0.1 M KOH in dry methanol for 1 min at room temperature, with two major products being obtained. The less polar and major product, 5-*O*-[(*Z*)-2-methyl-2-butenoyl]-16-*O*-acetyl-20-deoxy-16-hydroxyingenol (**19**, 3 mg), when extracted into chloroform and purified by preparative tlc in solvent 2 (Rf 0.49) was found to exhibit the following data: resin; $[\alpha]^{25}D$ −24.2° (c 0.155, $CHCl_3$); uv, λ max 21 nm (log ε 4.18); ir, ν max ($CHCl_3$) 3500, 2950, 1710, 1440, 1370, 1240, and 1220 cm^{-1}; pmr, (60 MHz) δ 0.98 (3H, d, J=7.0 Hz, 18-CH_3), 1.13 (3H, s, 17-CH_3), 1.56 (3H, s, 20-CH_3), 1.82 (3H, d, J=1.0 Hz, 19-CH_3), 1.95, 2.07 (3H each, m, 4′-CH_3, 5′-CH_3), 2.07 (3H, s, -OCOCH_3), 3.74 (1H, s, 3-H), 4.16, 4.36 (2H, AB_q, J_{AB}=12.0 Hz, 16-H_2), 5.24 (1H, br s, 5-H), 5.83 (1H, d, J=2.8 Hz, 7-H), 5.96 (1H, d, J=1.1 Hz, 1-H), and 6.12 ppm (1H, m, 3′-H); ms, *m/z* 472 (M^+, 0.5%), 454 (1), 413 (1), 412 (1), 394 (2), 372 (2), 371 (3), 354 (4), 330 (1), 329 (3), 312 (6), 294 (6), 284 (4), 279 (4), 251 (5), 189 (7), 162 (11), 151 (13), 135 (11), 122 (14), 121 (13), 95 (19), and 83 (100).

The more polar product, 5-*O*-[(*Z*]-2-methyl-2-butenoyl]-20-deoxy-16-hydroxyingenol (**20**, 1.5 mg), on purification by preparative tlc in solvent 2 (Rf 0.18), exhibited the following data: resin: $[\alpha]^{25}D$ −18.0° (c 0.025, $CHCl_3$); uv, λ max 212 nm (log ε 4.05); ir, ν max (AgCl) 3425, 2925, 1713, 1445, 1375, and 1225 cm^{-1}; pmr, (60 MHz) δ 0.99 (3H, d, J=6.7 Hz, 18-CH_3), 1.17 (3H, s, 17-CH_3), 1.57 (3H, br s, 20-CH_3), 1.83 (3H, d, J=1.2 Hz, 19-CH_3), 1.95, 2.06 (3H each, m, 4′-CH_3, 5′-CH_3), 2.22-2.42 (1H, m, exchangeable with D_2O, -OH), 3.77 (1H, s, 3-H), 3.82 (2H, s, 16-H_2), 4.25 (1H, m, 8-H), 5.24 (1H, br s, 5-H), 5.85 (1H, m, 7-H), 5.96 (1H, d, J=1.5 Hz, 1-H) and 6.18 ppm (1H, m, 3′-H); ms, *m/z* 430 (M^+, 1%), 412 (2), 394 (1), 330 (9), 329 (5), 312 (13), 294 (3), 253 (8), 217 (14), 189 (19), 162 (24), 151 (32), 135 (40), 123 (51), 122 (58), 121 (39), 95 (41), and 83 (100).

When **5** (1 mg) was hydrolyzed in 0.5 M KOH in dry methanol for 30 min, and acetylated and worked up in a similar manner to that described for **1**, 20-deoxy-16-hydroxyingenol-3,5,16-triacetate (**21**, 0.5 mg) was produced, which was identified by comparison (mp, ms, co-tlc) with a sample of this compound obtained in earlier work on *E. hermentiana* latex (6).

HYDROLYSIS EXPERIMENTS ON 3-*O*-TETRADECANOYLINGENOL (**22**).—A sample (1 mg) of the commercially available ingenol monester, 3-*O*-tetradecanoylingenol[6] (**22**), exhibited the following pmr characteristics at 60 MHz, δ 0.96 (3H, d, J=7.8 Hz, 18-CH_3), 1.05, 1.09 (3H each, s, 16-CH_3, 17-

[6]Consolidated Midland Corporation, Brewster, NY.

CH_3), 1.26 (22H, s, acyl methylene H), 1.77 (3H, br s, 19-CH_3), 2.29 (2H, m, -CH_2CO), 2.45 (2H, m, 12-H_2), 3.54 (1H, m, exchangeable with D_2O, -OH), 4.02 (1H, br s, 5-H), 4.12 (2H, s, 20-H_2), 4.23 (1H, m, 8-H), 5.50 (1H, s, 3-H), and 6.05 ppm (2H, m, 1-H, 7-H).

When **22** (3 mg) was hydrolyzed in 0.02 M KOH in methanol for 2 min at room temperature, none of the starting material remained. However, purification of the less polar and major of two reaction products by preparative tlc in solvent 2 (Rf 0.45) afforded 20-*O*-tetradecanoylingenol (**23**, 1.5 mg), which exhibited the following pmr spectral data at 60 MHz, δ 0.96 (3H, d, J=8.1 Hz, 18-CH_3), 1.06, 1.11 (3H each, s, 16-CH_3, 17-CH_3), 1.26 (22H, s, acyl methylene H), 1.84 (3H, br s, 19-CH_3), 2.19 (2H, m, -CH_2O), 2.36 (2H, m, 12-H_2), 2.97, 3.14 (2H, m, exchangeable with D_2O, -OH), 3.56 (1H, br s, 5-H), 4.10 (1H, m, 8-H), 4.11 (1H, m, exchangeable with D_2O, -OH), 4.38 (1H, s, 3-H), 4.43, 4.68 (2H, AB_q, J_{AB}= 10.0 Hz, 20-H_2), 5.92 (1H, d, J= 1.8 Hz, 1-H), and 6.07 ppm (1H, d, J=5.6 Hz, 7-H). Compounds **23** and **22** were separable by analytical tlc in solvents 1 (Rf 0.20 vs. 0.12), 2 (Rf 0.45 vs. 0.33), and 3 (Rf 0.08 vs. 0.04), respectively.

A portion of **22** (0.5 mg) was treated with Dowex 50W-X2[5] resin, under the same conditions used to hydrolyze compound **2**. Essentially no conversion to either 20-*O*-tetradecanoylingenol (**23**) or to any other reaction product was observed on tlc analysis after work up.

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