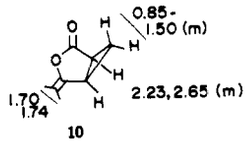
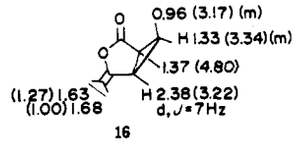
methyls. Registry no.: **9**, 15973-50-9; **13**, 63449-08-1; **14**, 63526-14-7; **20**, 63449-09-2; **21**, 63449-10-5; **13a**, 63449-11-6.

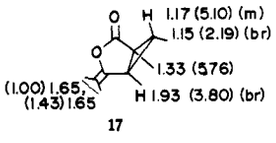
(32) The γ-lactones mentioned in this paper have the following NMR spectra (all peaks whose multiplicity is not indicated are singlets), where isomeric pair **16** and **17** can be distinguished by the Eu-shift slopes and chemical shifts of the C(6) hydrogens and methyls, and isomeric pair **17** and **17a** can be distinguished by similar examination of the C(1) and C(5) substituents. Registry no.: **10**, 29980-22-1; **16**, 63449-12-7; **17**, 63526-15-8; **17a**, 63449-13-8; **10a**, 63449-14-9.



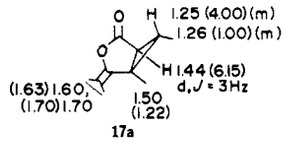
10



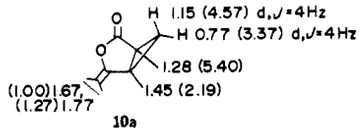
16



17



17a



10a

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Synthesis of 4aα-Phorbol 9-Myristate 9a-Acetate and Related Esters

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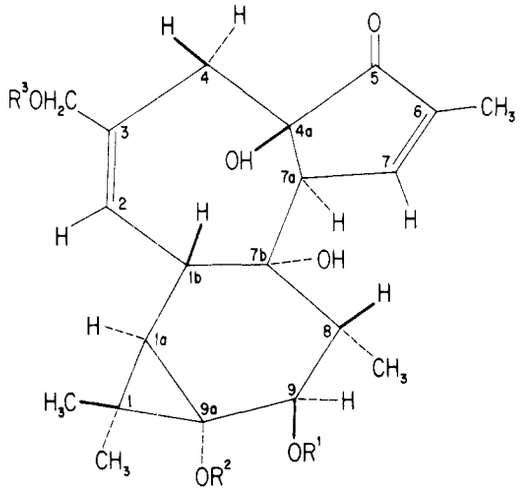
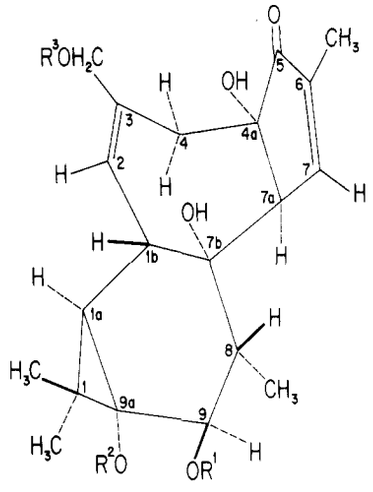
The stereoisomer of the most potent tumor-promoting agent phorbol 9-myristate 9a-acetate and its analogues have been synthesized and fully characterized. Phorbol isolated from croton oil was epimerized with 0.1 N sodium methoxide to 4aα-phorbol. Selective base-catalyzed esterification of 4aα-phorbol and selective acid-catalyzed hydrolysis of the resulting esters gave the desired compounds. They are: 4aα-phorbol 9-myristate 9a-acetate, 4aα-phorbol 9,9a-didecanoate, 3-decanoyl-4aα-phorbol 9,9a-didecanoate, 3-acetyl-4aα-phorbol 9,9a-diacetate, 3-acetyl-4aα-phorbol 9a-acetate, 4aα-phorbol 9a-acetate, 3-acetyl-4aα-phorbol 9-myristate 9a-acetate, 3-myristoyl-4aα-phorbol 9-myristate 9a-acetate, and 3-myristoyl-4aα-phorbol 9a-acetate.

Croton oil is a complex lipid mixture obtained by extraction or expression of the seeds of *Croton tiglium L.* This oil was first discovered to be a tumor promoter on mouse skin in two-stage carcinogenesis by Berenblum.[1] The subject of tumor promoters and cocarcinogens was recently reviewed.[2] The active principles of croton oil were isolated and characterized as the esters of the tetracyclic diterpene alcohol, phorbol[3,4] (**1a**). The structure and stereochemistry of **1a** was established from x-ray crystallographic studies.[5,6] Partial syntheses of phorbol esters have been reported.[7] We now wish to report the synthesis of 4aα-phorbol 9-myristate 9a-acetate (**2b**), which is the most important counterpart of the potent tumor promoter phorbol 9-myristate 9a-acetate[8] (**1b**), and the related



1a. $R^1=R^2=R^3=H$
b. $R^1=CO(CH_2)_{12}CH_3$; $R^2=COCH_3$; $R^3=H$
c. $R^1=R^2=CO(CH_2)_8CH_3$; $R^3=H$



2a. $R^1=R^2=R^3=H$
b. $R^1=CO(CH_2)_{12}CH_3$; $R^2=COCH_3$; $R^3=H$
c. $R^1=R^2=CO(CH_2)_8CH_3$; $R^3=H$
d. $R^1=R^2=R^3=CO(CH_2)_8CH_3$
e. $R^1=R^2=R^3=COCH_3$
f. $R^1=H$; $R^2=R^3=COCH_3$
g. $R^1=R^3=H$; $R^2=COCH_3$
h. $R^1=CO(CH_2)_{12}CH_3$; $R^2=R^3=COCH_3$
i. $R^1=R^3=CO(CH_2)_{12}CH_3$; $R^2=COCH_3$
j. $R^1=H$; $R^2=COCH_3$; $R^3=CO(CH_2)_{12}CH_3$

esters: 4aα-phorbol 9,9a-didecanoate (**2c**), 3-decanoyl-4aα-phorbol 9,9a-didecanoate (**2d**), 3-acetyl-4aα-phorbol 9,9a-diacetate (**2e**), 3-acetyl-4aα-phorbol 9a-acetate (**2f**), 4aα-phorbol 9a-acetate (**2g**), 3-acetyl-4aα-phorbol 9-myristate 9a-acetate (**2h**), 3-myristol-4aα-phorbol 9-myristate 9a-acetate (**2i**), and 3-myristol-4aα-phorbol 9a-acetate (**2j**). Com-

Table I. Selective NMR Data for Phorbol, 4aα-Phorbol, and Esters[a]

	Registry no.	1a	1b	2	3-CH_2	3-CH_2OR	4	7	9	9a
1a	17673-25-5	0.56 (1 H) d, J = 5	2.92 (1 H) m	5.50 (1 H) d, J = 6	3.80 (2 H) d, J = 5	4.70 (OH) t, J = 5	2.32 (2 H) s	7.58 (1 H) br s	4.06 (1 H) m; 4.30 (OH) d, J = 5	3.90 (OH) s
2a	26241-63-4	0.35 (1 H) d, J = 5	1.52–1.82 (1 H) m	5.12 (1 H) br s	3.55 (2 H) d, J = 5	4.66 (OH) br s	1.95 (1 H) d, J = 14; 3.23 (1 H) d, J = 14	7.30 (1 H) br s	4.06 (1 H) d, J = 7; 4.58 (OH) br s	4.02 (OH) s
2b	63597-44-4	0.80 (1 H) d, J = 5	1.50–2.00 (1 H) m	5.25 (1 H) br s	3.92 (2 H) s	2.85 (OH) s	2.31 (1 H) d, J = 14; 3.74 (1 H) d, J = 14	7.06 (1 H) br s	0.85 (3 H) t, J = 7; 1.28 (22 H) br s; 1.50–2.00 (2 H) m; 5.50 (1 H) d, J = 10	2.06 (3 H) s
2c	27536-56-7	0.81 (1 H) d, J = 5	1.50–2.00 (1 H) m	5.25 (1 H) br s	3.95 (2 H) s	2.33 (OH) s	2.30 (1 H) d, J = 14; 3.73 (1 H) d, J = 14	7.05 (1 H) br s	0.85 (3 H) t, J = 7; 1.26 (14 H) br s; 1.50–2.00 (2 H) m; 5.48 (1 H) d, J = 10	0.85 (3 H) t, J = 7; 1.26 (14 H) br s; 1.50–2.00 (2 H) m
2e	22338-57-4	0.86 (1 H) d, J = 5	1.60–2.00 (1 H) m	5.29 (1 H) br s	4.32 (2 H) s	2.12 (3 H) s	2.30 (1 H) d, J = 14; 3.70 (1 H) d, J = 14	7.05 (1 H) br s	2.04 (3 H) s; 5.43 (1 H) d, J = 10	2.08 (3 H) s
2f	63640-08-4	0.85 (1 H) d, J = 6	1.62–2.00 (1 H) m	5.30 (1 H) br s	4.32 (2 H) s	2.08 (3 H) s	2.26 (1 H) d, J = 14; 3.55 (1 H) d, J = 14	7.03 (1 H) br s	3.48 (OH) s; 4.03 (1 H) d, J = 10	2.06 (3 H) s
2g	63640-19-7	0.90 (1 H) d, J = 6	1.62–2.00 (1 H) m	5.30 (1 H) br s	3.93 (2 H) s	2.41 (OH) s	2.38 (1 H) d, J = 14; 3.65 (1 H) d, J = 14	7.13 (1 H) br s	3.55 (OH) s; 4.10 (1 H) d, J = 10	2.10 (3 H) s
2h	63588-55-6	0.81 (1 H) d, J = 6	1.50–2.00 (1 H) m	5.29 (1 H) br s	4.33 (2 H) s	2.12 (3 H) s	2.30 (1 H) d, J = 14; 3.72 (1 H) d, J = 14	7.03 (1 H) br s	0.85 (3 H) t, J = 7; 1.26 (22 H) br s; 1.50–2.00 (2 H) m; 5.38 (1 H) d, J = 10	2.08 (3 H) s
2i	63533-70-0	0.81 (1 H) d, J = 6	1.50–2.00 (1 H) m	5.29 (1 H) br s	4.35 (2 H) s	0.85 (3 H) t, J = 7; 1.26 (22 H) br s; 150–2.00 (2 H) m	2.30 (1 H) d, J = 14; 3.70 (1 H) d, J = 14	7.05 (1 H) br s	0.85 (3 H) t, J = 7; 1.26 (22 H) br s; 1.50–2.00 (2 H) m; 5.50 (1 H) d, J = 10	2.08 (3 H) s
2j	63533-71-1	0.82 (1 H) d, J = 6	1.50–2.00 (1 H) m	5.30 (1 H) br s	4.33 (2 H) s	0.85 (3 H) t, J = 7; 1.27 (22 H) br s; 1.50–2.00 (2 H) m	2.31 (1 H) d, J = 14; 3.72 (1 H) d, J = 14	7.04 (1 H) br s	2.40 (OH) s; 4.05 (1 H) d, J = 10	2.06 (3 H) s

[a] Spectra were run in $CDCl_3$ solutions (unless otherwise stated) at 60 MHz with Me_4Si as internal standard. Coupling constants are in hertz and chemical shifts values are in units of δ (ppm). Multiplicity: d, doublet; m, multiplet; s, singlet; br s, broadened singlet; t, triplet. [b] Spectra were taken in Me_2SO-d_6.

pounds **2c, 2d,** and **2e** have been reported in the literature,[9,10] but no synthesis or characterization was given for **2c** and **2d.** The availability of 4aα-phorbol esters described in this paper provides additional important molecules for studying the structure–activity relationships and the mechanisms of tumor promotion.

The starting compound **1a** was isolated and purified from croton oil according to the procedures previously described.[11-14] To achieve the epimerization at the 4a position, **1a** was treated with 0.1 N sodium methoxide in methanol at room temperature for 2 days. Pure **2a** was isolated in 72% yield after column chromatography and recrystallization. This compound is hygroscopic; therefore, crystallization was performed in a drybox. Elemental analysis and chemical ionization mass spectrometry, with isobutane as reagent gas, confirmed the molecular formula $C_{20}H_{28}O_6$. The IR spectrum and mass spectrometric fragmentation pattern were essentially identical with that of **1a.** However, in the UV spectrum the 234-nm peak of phorbol was shifted to 240 nm. Dreiding molecular models show that **1a** has a rigid structure. The cyclopentenoyl ring is trans connected with the unsaturated seven-membered ring. Because the latter is fixed in an envelope conformation with the fold between C-4a and C-1b, it imposes the strain on the former, and the cyclopentenoyl ring is not planar. In **2a,** where the 4a-hydroxyl group is in the α configuration, the cyclopentenoyl ring is cis connected to the seven-membered ring. The latter has more flexibility, with the fold through C-4 and C-7b. This allows the cyclopentenoyl ring to achieve the coplanarity. The NMR spectrum also showed some proton absorption peaks characteristic of this stereochemistry. The absorption peak of the methine proton at the C-1b position in **1a** was shifted downfield to δ 2.92 due to its steric interaction with the 4aβ-hydroxyl group; however, the same methine proton in **2a** is free of hydrogen bonding and showed the usual absorption peaks at δ 1.52–1.82 region. On the other hand, one of the C-4 methylene protons in **2a** is hydrogen bonded to the C-7b hydroxyl group; these two protons are not equivalent and showed two doublets at δ 1.95 and 3.23 with the typical geminal proton coupling constant of 14 Hz. In **1a,** these two protons are equivalent and free of hydrogen bonding, thus giving only a singlet peak at δ 2.32. The TLC also distinguished **1a** and **2a,** the R_f values were 0.6 and 0.5, respectively, in the solvent system of methanol–chloroform (1:3). On spraying the TLC plates with vanillin–sulfuric acid solution in ethanol and heating, **1a** showed a light green color while **2a** showed a dark green color. The stereoisomer of **1a** had been reported as 4α-phorbol by Hecker and his coworkers;[10,15] however, they could not obtain the crystalline form and this compound was isolated as the corresponding triacetate.[10,15] When **2a** was treated with acetic anhydride in dry pyridine under a nitrogen atmosphere, at room temperature, **2e** was isolated. Its melting point and IR, UV, and NMR spectra were identical with that of 4α-phorbol triacetate reported by Jacobi et al.[10] The NMR data of relevant compounds are given in Table I.

The difference in reactivity among five hydroxyl groups in **2a** provides the opportunity for selective esterification. There are three tertiary, one secondary, and one primary hydroxyl groups in the molecule. The tertiary hydroxyl group at C-9a is attached to a cyclopropane ring. It may be considered as a "homoenol" and should show the similar chemical reactivity compared with the ordinary enol.[16] In fact, when **2a** was treated with acetic anhydride in pyridine, **2f** was also isolated as the minor product. The difference in reactivity of the three hydroxyl groups of **2a** may be partially explained in terms of steric and conformational factors. The C-9 is probably more sterically hindered by the C-9 methyl group than is the C-9a hydroxyl group by either methyl group attached at C-1. Similarly, the primary hydroxyl group might be hydrogen bonded with the carbonyl at C-5. When milder conditions were used, in which the reaction was conducted in acetic anhydride and dimethylformamide in the presence of calcium carbonate, the diacetate **2f** and monoacetate **2g** were obtained, but **2e** was not isolated. This indicates that C-9a–OH of **2a** was acetylated even more rapidly than the hydroxyl group at the 3-methyl position. Similar results had been observed for **1a** by Szczepanski et al.[17] Under these weak basic and oxygen-free conditions, products due to the opening of the cyclopropane ring in **2a** were not isolated. Elemental analysis and chemical ionization mass spectrometry indicate molecular formulas $C_{24}H_{32}O_8$ and $C_{22}H_{30}O_7$ for **2f** and **2g,** respectively; moreover, mass spectrometric fragmentation patterns and NMR peak intensity for the methyl protons also confirmed the number of acetyl groups in these molecules. IR spectra of both acetates showed the characteristic ester carbonyl band at 1740 cm^{-1}. The UV spectra of both **2f** and **2g** were similar to that of the parent **2a.** In the NMR spectra, the downfield chemical shift (δ 4.32) of the C-3 methylene protons in **2f** indicated that one acetyl group was attached to the 3-hydroxymethyl position. By comparing the chemical shifts of the C-9 proton in **2e** (δ 5.45), **2f** (δ 4.03), and **2g** (δ 4.10), it can be concluded that C-9–OH was not acetylated and that the other acetyl group must be attached to the C-9a position in both **2f** and **2g.**

The next step in the synthesis of **2b** was then conducted by the esterification at the C-9 position. It was found that the esterification proceeded very slowly when **2f** or **2g** in pyridine was stirred with myristoyl chloride at room temperature. Much better results were obtained when the reactions were conducted in pyridine–toluene solutions and the mixtures heated to 90 °C. Under these conditions **2f** gave **2h,** and **2g** gave **2i** and **2j.** These three compounds were isolated by chromatography, and their structures were confirmed by UV, IR, NMR, and mass spectrometry. Finally, acid-catalyzed hydrolysis of both **2h** and **2i** with 60% perchloric acid[10] selectively removed the ester group at the C-3 hydroxymethyl position to yield **2b.** This selective hydrolysis is probably due to the accessibility and reactivity of the primary allylic hydroxyl, which may also be relevant to the acetylation reactions discussed above.

Our original attempts to obtain molecular weight information for **2b** and the other 4aα-phorbol esters by electron impact mass spectrometry were not successful; only the low mass fragmentation peaks were detected. By changing to the chemical ionization mode with isobutane as reagent gas, however, we were able to obtain not only molecular weights but also fragmentation peaks indicating the number and the kind of ester present in the molecules. In **2b,** the molecular weight (616) was clearly indicated by the protonated molecular ion peak (*m/e* 617 $(M + H)^+$). The fragmentation peaks at *m/e* 557 $(M + H - CH_3COOH)^+$ and 389 $[M + H - CH_3(CH_2)_{12}COOH]^+$ showed that **2b** contained one acetate group and one myristate group. Furthermore, the mass spectrum also showed three peaks (*m/e* 329, 311, and 293) characteristic of the phorbol molecule. Similar spectra were obtained for all the other 4aα-phorbol esters. A more detailed analysis of the chemical ionization mass spectra of 4aα-phorbol esters will be published elsewhere.[18] Elemental analysis confirmed that **2b** has the molecular formula $C_{36}H_{56}O_8$. Its IR spectrum was essentially identical with that of the pure **1b** isolated from croton oil in this laboratory.[19] However, the UV spectra of **1b** and **2b** (Figure 1) were significantly different; the longer wavelength band in **2b** was more pronounced than that in **1b** and this band was shifted to a yet longer wavelength, thus indicating that **2b** is a stereoisomer of **1b** with C-4a–OH oriented in the α configuration as discussed above. The NMR spectrum also showed proton absorption peaks characteristic of **2b,** the methylene protons

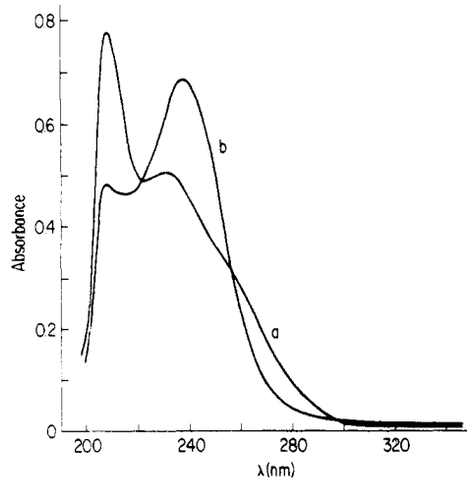


Figure 1. UV spectra of phorbol 9-myristate 9a-acetate (**1b**) (a) and 4aα-phorbol 9-myristate 9a-acetate (**2b**) (b) in ethanol, both at 1×10^{-4} M.

at the C-4 position showed two doublets at δ 2.30 and 3.65 (J = 14 Hz) due to one of the protons which was hydrogen bonded to C-7b–OH. The C-7b hydroxyl apparently exerts a deshielding effect on one C-4 proton because of close proximity. The TLC R_f value of **2b** (0.31) was lower than that of **1b** (0.44) using acetone–methylene chloride (1:6) as eluent. Compound **2b** was obtained as a viscous oil. The diester **2c** was prepared by the acid-catalyzed hydrolysis of **2d,** which in turn was synthesized from **2a** and decanoyl chloride in pyridine. For comparison, phorbol 9,9a-didecanoate (**1c**) was also synthesized from **1a** in the same manner. The structures of **2c** and **1c** were fully characterized.

Long-term in vivo two-stage carcinogenesis experiments with these 4aα-phorbol esters are currently underway in this laboratory in order to evaluate the effects of stereochemical configuration on tumor-promoting activity.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 137 spectrophotometer; samples were run in potassium bromide pellets or in carbon tetrachloride solutions. UV spectra were recorded on a Beckman Model 25 spectrophotometer. NMR spectra were recorded on a Varian Associates Model T-60A spectrometer in deuteriochloroform and deuteriodimethyl sulfoxide using tetramethylsilane as an internal standard. For the convenience of the reader all NMR data are summarized in Table I. Mass spectra were recorded on a DuPont 21-492 computer-based double-focusing high-resolution mass spectrometer in the chemical ionization mode using isobutane as reagent gas.[18] Analytical TLC was run on EM Reagents precoated TLC plates silica gel 60F-254 with fluorescent indicator. Spots were detected by UV light (254 nm) and by spraying the plates with a vanillin (3 g)–sulfuric acid (0.5 mL) solution in ethanol (100 mL), followed by heating. Column chromatography was carried with EM Reagents silica gel 60 (70-230 mesh ASTM) and neutral Florisil[19] (60–100 mesh; Fisher Scientific Co., New York, N.Y.). Croton oil was purchased from Amend Drug Company, Hillside, N.J. Microanalysis were performed by Galbraith Laboratories, Knoxville, Tenn.

Isolation and Purification of Phorbol (1a). The procedures of Flaschenträger[11,12] and Kauffmann[13,14] were used to isolate **1a** from croton oil. Compound **1a** crystallized with 1 mol of ethanol; 5.97 g of this material was obtained from 450 g of croton oil after recrystallization from ethanol (yield, 1.3%): mp 248–250 °C dec (lit.[20] 249–250 °C dec); IR (KBr) 3400 (OH), 1710 (α,β-unsaturated C=O), 1640 cm^{-1} (C=C); TLC, R_f 0.60 (CH_3OH–$CHCl_3$, 1:3).

Anal. Calcd for $C_{20}H_{28}O_6 \cdot C_2H_5OH$: C, 64.35; H, 8.35. Found: C, 64.18; H, 8.48.

Compound **1a**·C_2H_5OH was unstable; upon standing at 5 °C for several days the TLC of this compound showed several other spots. **1a**·C_2H_5OH (5 g) was dissolved in 100 mL of water and allowed to stand for 15 min at 60 °C. Water was removed in a rotatory evaporator (temperature bath, 60 °C) until crystals began to separate. This preparation was kept in the cold room for 1 week, and the crystals formed were collected by suction and dried under vacuum (4.40 g): mp 230–231 °C dec.

Anal. Calcd for $C_{20}H_{28}O_6 \cdot H_2O$: C, 62.82; H, 7.85. Found: C, 62.78; H, 7.80.

Pure **1a** (4.35 g) was obtained by azeotropic distillation of the water with benzene and drying under high vacuum for 24 h at 100 °C: mp 250–251 °C dec (lit.[20] 250–251 °C dec); TLC, R_f 0.6 (CH_3OH–$CHCl_3$, 1:3); IR (KBr) 3501, 3350, 1710, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 210 (ϵ 7343), 234 (5117), 332 nm (70); mass spectrum, m/e 365 $(M + H)^+$.

Anal. Calcd for $C_{20}H_{28}O_6$: C, 65.93; H, 7.75. Found: C, 66.08; H, 7.66.

4aα-Phorbol (2a). To a solution of **1a** (1.0 g, 2.7 mmol) in 30 mL of methanol under a nitrogen atmosphere was added 30 mL of 0.2 N sodium methoxide in methanol. A pink color developed immediately, which turned yellow after 30 min. The solution was stirred at room temperature in the dark for 2 days and then evaporated to dryness in vacuo. Column chromatography (silica gel) using methanol–chloroform (1:9, 1:6, and 1:3) gave **2a** (0.72 g, 72%). This compound is hygroscopic; it was recrystallized from ethyl acetate in a drybox: mp 135–150 °C; TLC, R_f 0.5 (CH_3OH–$CHCl_3$, 1:3), dark green color with vanillin spray; IR (KBr) 3450, 1710 (α,β-unsaturated C=O), 1640 cm^{-1} (C=C); UV (C_2H_5OH) λ_{max} 204 (ϵ 4568), 240 (5327), 334 nm (85); mass spectrum, m/e 365 $(M + H)^+$.

Anal. Calcd for $C_{20}H_{28}O_6$: C, 65.93; H, 7.75. Found: C, 66.11; H, 7.65.

3-Acetyl-4aα-phorbol 9,9a-Diacetate (2e). Acetic anhydride (0.1 mL) was added to a solution of **2a** (0.05 g, 0.14 mmol) in dry pyridine (2 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred at room temperature for 24 h. To this mixture was added 10 mL of water and 10 mL of methylene chloride. The water layer was further extracted with methylene chloride (2 × 10 mL). The organic layer was acidified with 1 N HCl, neutralized with 5% $KHCO_3$, washed with saturated NaCl, and then dried over $MgSO_4$. Evaporation of the solvent afforded 0.065 g of crude solid product. TLC (acetone–methylene chloride, 1:3) showed two spots with R_f values 0.73 and 0.30. Column chromatography (silica gel) with acetone–methylene chloride (1:9) as eluent gave 0.050 g (73%) of **2e** (TLC, R_f (0.73)). This product was recrystallized from ether–petroleum ether: mp 171–173 °C (lit.[10] 172–175 °C); IR (KBr) 3550, 3400, 2900, 1740 (ester C=O), 1720, 1710, 1630 cm^{-1}; UV (C_2H_5OH) λ_{max} 205 (ϵ 5050), 240 (6784), 334 nm (110); mass spectrum, m/e 491 $(M + H)^+$.

Anal. Calcd for $C_{26}H_{34}O_9$: C, 63.67; H, 6.93. Found: C, 63.75; H, 6.81.

Another 0.010 g of a white solid (TLC, R_f 0.30) was obtained from column chromatography. This material was identified as **2f** as described below.

3-Acetyl-4aα-phorbol 9a-Acetate (2f) and 4aα-Phorbol 9a-Acetate (2g). Acetic anhydride (1.8 mL) and $CaCO_3$ (1.8 g) were added to a solution of **2a** (0.88 g, 2.4 mmol) in dry dimethylformamide (18 mL) with stirring at room temperature under a nitrogen atmosphere. The resulting suspension was stirred at room temperature for 24 h, at which time another 3.6 mL of acetic anhydride and 3.6 g of $CaCO_3$ were added. After 24 h the mixture was poured into 80 mL of water. The aqueous solution was then extracted with ethyl acetate (3 × 80 mL). The extracts were washed with 50 mL of 5% $KHCO_3$ and then with 50 mL of saturated NaCl. The ethyl acetate layer was dried over $MgSO_4$. Evaporation of this solution gave 0.87 g of crude solid product. TLC showed two major spots with R_f values of 0.30 and 0.10 using acetone–methylene chloride (1:3) as eluent. Column chromatography (silica gel) of the crude products with ether and ether–ethyl acetate (6:1) as eluents gave 0.45 g of **2f** (42%) and 0.30 g of the monoacetate **2g** (30%). Compound **2f** was recrystallized from ether: mp 182–184 °C; TLC, R_f 0.30 (acetone–methylene chloride, 1:3); IR (KBr) 3550, 3400, 2900, 1740 (ester C=O), 1720, 1710, 1630 cm^{-1}; UV (C_2H_5OH) λ_{max} 205 (ϵ 4552), 240 (6552), 334 nm (100); mass spectrum, m/e 449 $(M + H)^+$.

Anal. Calcd for $C_{24}H_{32}O_8$: C, 64.29; H, 7.74. Found: C, 64.13; H, 7.33.

Compound **2g** was recrystallized from ethyl acetate: mp 223–225 °C; TLC, R_f 0.10 (acetone–methylene chloride, 1:3); IR (KBr) 3550, 3400, 2950, 1740 (ester C=O), 1720, 1710, 1630 cm^{-1}; UV (C_2H_5OH) λ_{max} 204 (ϵ 4036), 240 (6330), 334 nm (91); mass spectrum, m/e 407 $(M + H)^+$.

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.02, H, 7.39. Found: C, 65.32; H, 7.56.

3-Acetyl-4aα-phorbol 9-Myristate 9a-Acetate (2h). To a solution of **2f** (0.10 g, 0.2 mmol) in pyridine (5 mL) and toluene (10 mL)

was added 0.1 g of myristoyl chloride. The mixture was stirred at 90 °C for 30 h. Evaporation of the toluene–pyridine solution in vacuo yielded a semisolid. It was washed with 5% $NaHCO_3$ (20 mL) and extracted with methylene chloride (3 × 20 mL), and then dried (Na_2SO_4). Evaporation of solvent gave the crude product, which after purification by column chromatography on Florisil (petroleum ether–ether, 2:1) yielded **2h** as a colorless oil (0.10 g, 77%): TLC, R_f 0.57 (acetone–methylene chloride, 1:6); IR (CCl_4) 3450, 2950, 2860, 1740, 1720, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 4750), 237 (6520), 334 nm (100); mass spectrum, *m/e* 659 $(M + H)^+$.

3-Myristoyl-4aα-phorbol 9-Myristate 9a-Acetate (2i) and 3-Myristoyl 4aα-phorbol 9a-Acetate (2j). Myristoyl chloride (0.3 g) was added to a solution of **2g** (0.15 g, 0.3 mmol) in dry pyridine (5 mL) and toluene (10 mL) at room temperature under a nitrogen atmosphere. The mixture was stirred at 90 °C for 20 h. Then the pyridine–toluene solution was evaporated in vacuo to give a semisolid residue. This residue was washed with 5% $NaHCO_3$ (20 mL), extracted with methylene chloride (3 × 30 mL), and dried over Na_2SO_4. Evaporation of solvent yielded the crude product. The TLC contained two major spots. Column chromatography of this crude product on neutral Florisil with petroleum ether–ether (1:1) as eluent gave **2i** as a semisolid (0.16 g, 63%): TLC, R_f 0.89 (acetone–CH_2Cl_2, 1:6); IR (CCl_4) 3450, 2950, 2860, 1740, 1720, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 7600), 234 (9090), 332 nm (450); mass spectrum, *m/e* 827 $(M + H)^+$.

Further column chromatography of the crude product with acetone–methylene chloride mixture (1:9) as eluent gave **2j** (0.05 g, 27%): TLC, R_f 0.39 (acetone–CH_2Cl_2, 1:6); IR (CCl_4) 3450, 2950, 2860, 1740, 1720, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 4390), 237 (6300), 334 (140); mass spectrum, *m/e* 617 $(M + H)^+$.

4aα-Phorbol 9-Myristate 9a-Acetate (2b). Two procedures were used. (a) To a solution of **2h** (0.10 g, 0.15 mmol) in methanol (10 mL) under a nitrogen atmosphere was added 0.1 mL of 60% perchloric acid. The mixture was stirred at room temperature for 20 h; 0.10 g of $NaOAc·3H_2O$ was then added to the methanol solution with stirring. After 10 min the solution was evaporated to dryness. Water (15 mL) and methylene chloride (15 mL) were added to the residue. The water layer was extracted with methylene chloride (2 × 20 mL). The combined organic layers were washed with 5% $NaHCO_3$ (15 mL) and saturated NaCl (15 mL), and then dried over Na_2SO_4. Evaporation of the solvent gave crude **2b** as an oil (0.08 g, 87%). It was purified by column chromatography on Florisil with acetone–methylene chloride (1:9) as eluent: TLC, R_f 0.31 (acetone–CH_2Cl_2 1:6); IR (CCl_4) 3450, 2950, 2860, 1740, 1720, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 4409), 237 (6236), 334 (140); mass spectrum, *m/e* 617 $(M + H)^+$.

Anal. Calcd for $C_{36}H_{56}O_8$: C, 70.13; H, 9.09. Found: C, 70.33; H, 9.00.

(b) To a solution of **2i** (0.10 g, 0.12 mmol) in methanol (10 mL) under a nitrogen atmosphere was added 0.1 mL of 60% perchloric acid. The mixture was stirred at room temperature for 24 h. Upon workup as described under (a) above, it gave the crude product, which, after purification by column chromatography, afforded pure **2b** (0.05 g, 68%). All spectral data were identical with that described under (a) above.

3-Decanoyl-4aα-phorbol 9,9a-Didecanoate (2d) and 4aα-Phorbol 9,9a-Didecanoate (2c). To a solution of **2a** (0.15 g, 0.41 mmol) in dry pyridine (5 mL) at 0 °C under a nitrogen atmosphere was added 0.55 g of decanoyl chloride. The mixture was stirred at room temperature for 3 days. To this mixture were added 30 mL of water and 30 mL of methylene chloride. The water layer was further extracted with methylene chloride (2 × 30 mL). The combined organic layers were acidified with 1 N HCl, neutralized with 5% $KHCO_3$, washed with saturated NaCl, and then dried over $MgSO_4$. Evaporation of the solvent gave a light yellow oil. Column chromatography (silica gel) with hexane–methylene chloride mixture (1:4) afforded pure **2d** as a colorless oil (0.23 g, 70%): TLC, R_f 0.90 (acetone–CH_2Cl_2, 1:6); IR (CCl_4) 3550, 3400, 2950, 2860, 1740, 1720, 1710, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 4960), 238 (6740), 334 nm (120); mass spectrum, *m/e* 827 $(M + H)^+$. To a solution of **2d** (0.20 g, 0.24 mmol) in methanol (15 mL) was then added 0.15 mL of 60% perchloric acid. After the mixture was stirred at room temperature for 20 h, $NaOAc·3H_2O$ (0.2 g) was added, and the solution evaporated to dryness. Water (30 mL) and methylene chloride (30 mL) were added to the residue. The water layer was further extracted with methylene chloride. The organic layers were washed with 5% $KHCO_3$ and saturated NaCl, and dried over $MgSO_4$. Evaporation of the solvent gave a yellow oil. Column chromatography on Florisil with acetone–methylene chloride (1:9) as eluent afforded pure **2c** as a colorless oil (0.10 g, 63%): TLC, R_f 0.26 (acetone–CH_2Cl_2, 1:6); IR (CCl_4) 3450, 2950, 2860, 1740, 1720, 1710, 1640 cm^{-1}; UV (C_2H_5OH) λ_{max} 207 (ε 4390), 238 (6440), 334 nm (120); mass spectrum, *m/e* 673 $(M + H)^+$.

Phorbol 9,9a-Didecanoate (1c). This compound was prepared from **1a** by the procedures described above for **2c**. Pure **1c** had the following physical properties: TLC, R_f 0.36 (acetone–CH_2Cl_2, 1:6); IR (CCl_4) 3400, 2950, 2860, 1740, 1720, 1710, 1635 cm^{-1}; UV (C_2H_5OH) λ_{max} 210 (ε 8560), 234 (5230), 332 nm (75); mass spectrum, *m/e* 673 $(M + H)^+$.

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Registry No.—**1b,** 16561-29-8; **1c,** 24928-17-4; **2d,** 63597-45-5; myristoyl chloride, 112-64-1; decanoyl chloride, 112-13-0.

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(8) We have used the trivial name phorbol myristate acetate since 1965.[3] The numbering of the benzazulene ring system of phorbol and the nomenclature used to describe its derivatives have differed among various authors.[15,20] The correct *Chemical Abstracts* numbering system is shown in **1** and **2.** This nomenclature has also been accepted by IUPAC. The correct *Chemical Abstracts* name for **1b** is as follows: 5*H*-cyclopropa[3,4]benz[1,2-*e*]-azulen-5-one, 1,1aα,1bβ,4,4a,7aα,7b,8,9,9a-decahydro-4aβ,7bα,9β,-9aα-tetrahydroxy-3-(hydroxymethyl)-1,1,6,8α-tetramethyl 9a-acetate 9-myristate (*Chemical Abstracts* Registry Number 16561-29-8). Because of the unwieldy length of the correct *Chemical Abstracts* nomenclature, we chose to use the trivial names for 4aα-phorbol and its esters in the title and the text.
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