Inhibition of Cytopathic Effect of Human Immunodeficiency Virus Type-1 by Various Phorbol Derivatives

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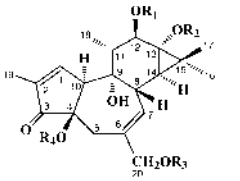
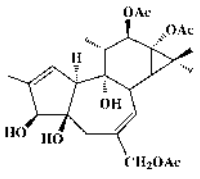
Forty-eight derivatives of phorbol (9) and isophorbol (14) were evaluated for their inhibition of human immunodeficiency virus (HIV)-1 induced cytopathic effects (CPE) on MT-4 cells, as well as their activation of protein kinase C (PKC), as indices of anti-HIV-1 and tumor promoting activities, respectively. Of these compounds, the most potent inhibition of CPE was observed in 12-*O*-tetradecanoylphorbol 13-acetate (8) and 12-*O*-acetylphorbol 13-decanoate (6). The former also showed the strongest PKC activation activity, while the latter showed no activity at 10 ng/ml. Both activities were generally observed in those phorbol derivatives with an A/B *trans* configuration, but not in the isophorbol derivatives with an A/B *cis* configuration. Acetylation of 20-OH in the phorbol derivatives significantly reduced the inhibition of CPE, as shown in 12-*O*-, 20-*O*-diacetylphorbol 13-decanoate (6a) (IC_{100}=15.6 μg/ml) *vs.* compound 6 (IC_{100}=0.0076 μg/ml), and 12-*O*-tetradecanoylphorbol 13,20-diacetate (8a) (IC_{100}=15.6 μg/ml) *vs.* 12-*O*-tetradecanoylphorbol 13-acetate (8) (IC_{100}=0.00048 μg/ml), except in the case of 12-*O*-decanoylphorbol 13-(2-methylbutyrate) (4) and phorbol 12,13-diacetate (9c). The reduction of a carbonyl group at C-3 abruptly reduced the inhibition of CPE, as observed in 3β-hydroxyphorbol 12,13,20-triacetate (9f) (IC_{100}=500 μg/ml) *vs.* phorbol 12,13,20-triacetate (9d) (IC_{100}=62.5 μg/ml). Although 8 was equipotent in the inhibition of CPE, and activation of PKC, both activities were abruptly decreased by the acetylation of 20-OH and methylation of 4-OH [as in 8a and 4-*O*-methyl-12-*O*-tetradecanoylphorbol 13,20-diacetate (8b), respectively]. On the other hand, its positional isomer (12-*O*-acetylphorbol 13-tetradecanoate (8c) showed neither activities. The removal of a long acyl group in 8 led to a substantial loss of both activities, as shown in phorbol 13-acetate (9b). Of the 12-*O*-acetyl-13-*O*-acylphorbol derivatives, the highest inhibition of CPE was observed in 6, which has a dodecanoyl residue at C-13. Both an increase and decrease in the number of fatty acid carbon chains resulted in significant reduction of the inhibition of CPE.

Key words human immunodeficiency virus; phorbol; protein kinase C; anti-HIV agent; 12-*O*-acetylphorbol 13-decanoate

Current therapy for human immunodeficiency virus (HIV) infection relies primarily on the administration of anti-retroviral nucleoside analogues, either alone or in combination with HIV-protease inhibitors. Although these drugs have a clinical benefit, continuous therapy with these drugs causes to creates drug-resistant strains of the virus. Recently, significant progress has been made towards the development of natural and synthetic agents that can directly inhibit HIV replication or its essential enzymes.[1—6] We previously reported the isolation of 8 phorbol diesters (**1**—**8**) from the seeds of *Croton tiglium* L., and 12-*O*-decanoylphorbol 13-*O*-(2-methylbutyrate) (**4**) and 12-*O*-acetylphorbol 13-decanoate (**6**) were found to potently inhibit an HIV-1-induced cytopathic effect (CPE) on MT-4 cells without the activation of protein kinase C (PKC) normally associated with tumor-promoting action.[7,8] This finding suggested that the development of **4** and **6** as lead compounds was a potential strategy for developing novel, therapeutically useful anti-HIV agents. In this paper, we report the chemical modification of phorbol (**9**) and isophorbol (**14**), and their biological activities focused on the inhibition of CPE and activation of PKC.



1a



9f

	R_1	R_2	R_3	R_4
1	H	Ac	$C_{18}H_{31}O$	H
1b	Ac	Ac	$C_{18}H_{31}O$	H
2	H	Tig	$C_{18}H_{31}O$	H
3	Ac	Tig	H	H
4	$C_{10}H_{19}O$	2-Me butyryl	H	H
4a	$C_{10}H_{19}O$	2-Me butyryl	Ac	H
5	Tig	2-Me butyryl	H	H
6	Ac	$C_{10}H_{19}O$	H	H
6a	Ac	$C_{10}H_{19}O$	Ac	H
6b	Ac	$C_{10}H_{19}O$	Ac	Me
7	2-Me butyryl	$C_{12}H_{23}O$	H	H
8	$C_{14}H_{27}O$	Ac	H	H
8a	$C_{14}H_{27}O$	Ac	Ac	H
8b	$C_{14}H_{27}O$	Ac	Ac	Me
8c	Ac	$C_{14}H_{27}O$	H	H
8d	Ac	$C_{14}H_{27}O$	$C_{14}H_{27}O$	H
9	H	H	H	H
9a	Ac	H	H	H
9b	H	Ac	H	H
9c	Ac	Ac	H	H
9d	Ac	Ac	Ac	H
9e	Ac	Ac	Ac	Me
9g	Ac	Ac	Ac	Ac
10	Bz	Bz	Bz	H
11	Ac	$C_6H_{11}O$	H	H
11a	Ac	$C_6H_{11}O$	$C_6H_{11}O$	H
12	Ac	$C_9H_{17}O$	H	H
12a	Ac	$C_9H_{17}O$	$C_9H_{17}O$	H
13	Ac	$C_{12}H_{23}O$	H	H
13a	Ac	$C_{12}H_{23}O$	$C_{12}H_{23}O$	H

Chart 1. Chemical Structures of Phorbol and Its Derivatives

Results

Synthesis of Phorbol and Isophorbol Derivatives Hydrolysis of a phorbol ester mixture from the seeds of *Croton tiglium* with $Ba(OH)_2$/MeOH[9,10] yielded tetracyclic diterpenes, phorbol (**9**), 4α-phorbol (**14**, isophorbol) and 4-deoxy-4α-phorbol (**23**), which were identified by comparison of their spectral data with those reported.[11—13] Phorbol

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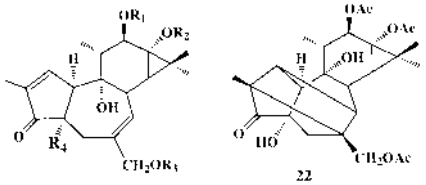
12,13,20-triacetate (**9d**) and phorbol 12,13,20-tribenzoate (**10**) were synthesized from **9**, while phorbol 12-acetate (**9a**), phorbol 12,13-diacetate (**9c**) and its 4-methyl ether (**9e**), 3β-hydroxyphorbol 12,13,20-triacetate (**9f**), and phorbol 4,12, 13,20-tetraacetate (**9g**) were from **9d** (Chart 1),[14—18] and isophorbol derivatives **14a**—**e** and **15**—**21** were from **14** and isophorbol 13-acetate (**14b**), respectively,[19] while a photo product **22** was obtained from isophorbol 12,13,20-triacetate (**14c**) after irradiation with UV light at 254 nm. On the other hand, 4-deoxy-4α-phorbol derivatives (**23a**—**c**) were prepared from **23**, while **24a**—**d** and **25** were obtained from **23b** (Chart 2).[19]



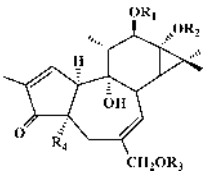
22

	R_1	R_2	R_3	R_4
14	H	H	H	OH
14a	H	Ac	H	OH
14b	H	Ac	Ac	OH
14c	Ac	Ac	Ac	OH
14d	C_4H_7O	C_4H_7O	C_4H_7O	OH
14e	Ac	Ac	Ac	OAc
15	$C_8H_{15}O$	Ac	Ac	OH
16	$C_{10}H_{19}O$	Ac	Ac	OH
17	10-Undecenoyl	Ac	Ac	OH
18	$C_{12}H_{23}O$	Ac	Ac	OH
19	$C_{14}H_{27}O$	Ac	Ac	OH
20	$C_{17}H_{33}O$	Ac	Ac	OH
21	1-Adamantanoyl	Ac	Ac	OH
23	H	H	H	H
23a	H	Ac	H	H
23b	H	Ac	Ac	H
23c	Ac	Ac	Ac	H
24	$C_{10}H_{19}O$	Ac	Ac	H
25	$C_{14}H_{27}O$	Ac	Ac	H

Chart 2. Chemical Structures of Isophorbol and Its Derivatives

Inhibition of HIV-1 Induced CPE and Activation of PKC Un-acylated phorbols (**9**, **14**, **23**) did not show any significant inhibition of HIV-induced CPE or activation of PKC (Tables 1 and 2). Most of the derivatives of isophorbol and 4-deoxy-4α-isophorbol (**14a**, **b**, **15**—**25**) were inactive

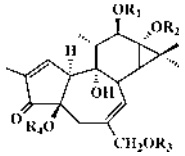
Table 1. Inhibition of HIV-1 Induced CPE and Activation of PKC by Isophorbol and Its Derivatives



No.	R_1	R_2	R_3	R_4	Inhibition of CPE (μg/ml)		% Activation of PKC[a]
					IC_{100}	CC_0	
14	H	H	H	OH	NE	500	0
14a	H	Ac	H	OH	NE	>1000	NT
14b	H	Ac	Ac	OH	NE	>1000	NT
14c	Ac	Ac	Ac	OH	250	500	0
14d	C_4H_7O	C_4H_7O	C_4H_7O	OH	NE	62.5	0
14e	Ac	Ac	Ac	OAc	NE	500	0
15	$C_8H_{15}O$	Ac	Ac	OH	31.25	500	NT
16	$C_{10}H_{19}O$	Ac	Ac	OH	125	500	NT
17	10-Undecenoyl	Ac	Ac	OH	125	500	NT
18	$C_{12}H_{23}O$	Ac	Ac	OH	NE	1000	NT
19	$C_{14}H_{27}O$	Ac	Ac	OH	NE	1000	NT
20	$C_{17}H_{33}O$	Ac	Ac	OH	NE	>1000	NT
21	1-Adamantanoyl	Ac	Ac	OH	250	500	NT
22					NE	500	0
23	H	H	H	H	NE	500	0
23a	H	H	Ac	H	62.5	1000	NT
23b	H	Ac	Ac	H	62.5	500	NT
23c	Ac	Ac	Ac	H	7.81	250	NT
24	$C_{10}H_{19}O$	Ac	Ac	H	31.25	1000	NT
25	$C_{14}H_{27}O$	Ac	Ac	H	62.5	1000	NT

a) At 10 ng/ml, relative to that shown by TPA (100% inhibition) (**8**); NE, not effective; NT, not tested.

Table 2. Inhibition of HIV-1 Induced CPE and Activation of PKC by Phorbol and Its Derivatives



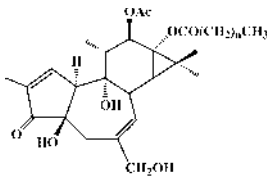
	R_1	R_2	R_3	R_4	Inhibition of CPE (μg/ml)		Activation of PKC	
					IC_{100}	CC_0	%[a]	MAC (μg/ml)
1	H	Ac	$C_{18}H_{31}O$	H	15.6	62.5	0	
1b	Ac	Ac	$C_{18}H_{31}O$	H	7.81	62.5	0	
2	H	Tig	$C_{18}H_{31}O$	H	7.81	62.5	14	
3	Ac	Tig	H	H	125	500	16	
4	$C_{10}H_{19}O$	2-Me butyryl	H	H	7.81	31.3	0	>50
4a	$C_{10}H_{19}O$	2-Me butyryl	Ac	H	3.90	15.6	10	>50
5	Tig	2-Me butyryl	H	H	31.3	62.5	10	
6	Ac	$C_{10}H_{19}O$	H	H	0.0076	62.5	0	>100
6a	Ac	$C_{10}H_{19}O$	Ac	H	15.6	31.3	11	
6b	Ac	$C_{10}H_{19}O$	Ac	Me	NE	1.95	0	
7	2-Me butyryl	$C_{10}H_{19}O$	H	H	15.6	62.5	16	
8	$C_{14}H_{27}O$	Ac	H	H	0.00048	31.3	100	0.01
8a	$C_{14}H_{27}O$	Ac	Ac	H	15.6	62.5	0	
8b	$C_{14}H_{27}O$	Ac	Ac	Me	NE	15.6	0	
8c	Ac	$C_{14}H_{27}O$	H	H	NE	125.0	0	
8d	Ac	$C_{14}H_{27}O$	$C_{14}H_{27}O$	H	62.5	125.0	0	
9	H	H	H	H	NE	1000	8	
9a	Ac	H	H	H	NE	500	13	
9b	H	Ac	H	H	125	>1000	0	
9c	Ac	Ac	H	H	NE	>1000	57	
9d	Ac	Ac	Ac	H	62.5	125	0	
9e	Ac	Ac	Ac	Me	31.3	125	0	
9g	Ac	Ac	Ac	Ac	125	250	0	
10	Bz	Bz	Bz	H	NE	31.3	100	

a) At 10 ng/ml, relative to that shown by TPA (100% inhibition) (**8**); MAC, minimum concn. for maximum activation of PKC; NE, not effective. Under the same conditions, dextrine sulfate DS 8000 (positive control) showed IC_{100} and CC_0 values of 3.90 and >1000, respectively.

(Table 1), while phorbol esters **9a**—**g** showed variable activities (Table 2). Of the isophorbol derivatives, 12-*O*-octanoylphorbol 13,20-diacetate (**15**), as well as mono- and diacetyl derivatives of **23** (**23a**, **b**), were moderately active against CPE (IC_{100} value of 62.5 μg/ml), and the activity of **23b** was remarkably enhanced by further acetylation (as in **23c**, IC_{100} value of 7.81 μg/ml). Decanoyl and tetradecanoyl derivatives of **23** (**24**, **25**) were moderately active (IC_{100} values of 31.25 and 62.5 μg/ml, respectively). As for the phorbol derivatives, 12-*O*-acetyl derivative (**9a**) was inactive, while the 13-*O*-acetyl counter part (**9b**) showed weak inhibition of CPE (Table 2). Although phorbol 12,13-diacetate (**9c**) and the tetraacetate (**9g**) were inactive, the triacetate (**9d**) was moderately active (IC_{100} value of 62.5 μg/ml). Methylation of the triacetate **9d** enhanced the inhibition of CPE (as in **9e**, IC_{100} value of 31.3 μg/ml), while reduction of a carbonyl group at C-3 sharply reduced the activity, as in **9f** (IC_{100} value of 500 μg/ml, CC_0 value of 1000 μg/ml, without PKC activation). A two-fold increase in the inhibitory activity against CPE was observed for **1a** (IC_{100} value of 7.81 μg/ml) by treatment of **1** with mesyl chloride. Similar enhancement of the inhibitory activity was also observed after introducing an acetyl group at C-12, as in 12-*O*-,13-*O*-diacetylphorbol 20-decanoate (**1b**).

Acetylation of **6** (as in **6a**) and 4-*O*-methylation (as in **6b**) significantly reduced the inhibitory activity against CEP, but enhanced the inhibitory activity of **4** by introducing an acetyl group at C-20 (as in **4a**, IC_{100} value of 3.9 μg/ml).

Table 3. Effects of Acyl Chain Length at C-13 of 12-*O*-Acetyl-13-*O*-acylphorbol Derivatives on Inhibition of HIV-1 Induced CPE and Activation of PKC



No.	*n*	Inhibition of CPE (μg/ml)		% Activation of PKC[a]
		IC_{100}	CC_0	
9c	0	NE	>1000	57
11	4	NE	62.5	27
12	7	31.3	31.3	10
6	8	0.0076	62.5	0
13	10	250	500	35
8c	12	NE	125	0

a) At 10 ng/ml, relative to that shown by TPA (100% inhibition) (**8**); NE, not effective.

Although **8** was found to be equipotent in terms of the inhibition of CPE and the activation of PKC, both activities were dramatically decreased by introducing an acetyl group at C-20 (as in **8a**) and by the methylation of a free hydroxyl group at C-4 (as in **8b**) (Table 2). On the other hand, its posi-

tional isomer (**8c**) was almost inactive. Removal of the long chain acyl group from **8** resulted in a substantial loss of both activities, as in **9b** (IC_{100} value of 125 μg/ml) (Table 2).

Table 3 shows the comparison of inhibitory activity of CPE and activation of PKC among various 12-*O*-acetyl-13-*O*-acylphorbols having different chain lengths of fatty acid residue (C6 : 0, C9 : 0, C12 : 0 and C14 : 0). The maximum inhibition of CPE was observed for compound **6** (C10 : 0), and both an increase and decrease in the chain length resulted in an abrupt decrease in the inhibitory activity against CPE. Similarly, the activation of PKC was appreciably influenced by an acyl group attached at C-13.

Discussion

The present study suggests that HIV-1 induced CPE inhibition and PKC-activation activities are influenced by the configuration of the diterpene ester, in which all active phorbol derivatives are of the A/B *trans* configuration. The A/B *cis* analogs (isophorbol type) showed no remarkable inhibitory effects on CPE. The finding that an anti-CPE activity of **1** was enhanced 2-times after homoallylic rearrangement of an α-(acetoxycyclopropyl)carbinol group (as in **1a**) suggested that this group is not a critical requirement for the anti-CPE activity of these compounds. The observation that TPA (**8**, with C_{14} and C_2 at C-12 and C-13, respectively) was equipotent in terms of both CPE inhibition and PKC activation, while **6** (with C_2 and C_{10} at C-12 and C-13) and **4** (with C_{10} and C_5 at C-12 and C-13) were potent inhibitors of HIV-1-induced CPE, but showed no activation of PKC, suggested that the difference in chain lengths of acyl groups and its relative positions significantly influenced both activities. Both activities were dramatically decreased by introducing an acetyl group at C-20 (as in **8a**), while removal of a long chain acyl group from **8** resulted in a substantial loss of both activities, as in **9b**.

Chowdhury *et al.*[21] found that the cocultivation of MOLT-4 and MOLT-4/HIV-$1_{HTLV\text{-}IIIB}$ cells at concentrations of more than 0.01 ng/ml of TPA (**8**) for 20 h strikingly inhibited HIV-induced syncytia formation through down-modulation of CD_4 molecules, and that these effects were abrogated by staurosporine, a PKC inhibitor. They reached the conclusion that TPA (**8**) acted through the activation of PKC in down-modulating CD_4 molecules and syncytia formation. Their findings are, in most respects, consistent with our findings that TPA (**8**) at a concentration of 0.4 ng/ml inhibited HIV-1induced CPE on MT-4 cells, and demonstrated the maximal activation of PKC. Phorbol and its esters (**9**, **9d**, **g**), which were previously reported as non-tumor promoters, were also very weak activators of PKC in the present experiment, though their CPE activity was variable.

Although there is strong biochemical evidence that PKC is the major receptor for phorbol esters,[22—29] the behavior of these compounds in biological systems clearly indicates that both activities cannot be explained by a single, homogenous class of well behaved receptors. For example, different phorbol derivatives showed different spectra of biological responses, and for a single compound, different concentrations may be required to induce different activities. Although **8** was an equipotent inhibitor of CPE and activator of PKC, **10**, which demonstrated the maximal activation of PKC, failed to inhibit CPE. The diesters 12-*O*-tigloylphorbol 13-decanoate isolated from *C. tiglium*,[30] and ostodin and 12-*O*-undecadienoylphorbol 13-acetate isolated from *Ostodes paniculata*,[31] were potent antitumor agents. 12-Deoxyphorbol 13-decadienoate, isolated from *Excoecaria agallocha* as an anti-HIV principle, was also a potent displacer of [^{3}H]-phorbol dibutyrate from rat brain membrane.[32] However, other derivatives (**4**, **6**) were found to potently inhibit the HIV-1-induced CPE without activating PKC. Kubinski *et al.*[33] reported that although chemically related compounds tend to change the properties of the microsomal membrane in a similar way, TPA (**8**) decreased the amount of bound DNA 5-fold, while phorbol 12,13-didecanoate, the second strongest promoter, increased this amount by about one-third. These discrepancies have been postulated to reflect a difference in the specific site of action, and led us to suggest that except for TPA (**8**), the inhibition of CPE and activation of PKC by these compounds are not parallel in potency, and that the anti-CPE activity shown by **4** and **6** is mediated through different receptor(s) other than/or in addition to PKC.

Experimental

Instruments Optical rotations were obtained on a DIP-360 automatic polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on an FT/IR-230 spectrophotometer (Jasco). UV spectra were measured with a UV-2200 UV-VIS recording spectrophotometer (Shimadzu, Kyoto, Japan). NMR spectra were obtained on a Varian Unity plus 500 (^{1}H-, 500 MHz; ^{13}C-, 125 MHz) spectrometer, and chemical shifts are given in δ ppm relative to tetramethylsilane (TMS). Electron impact (EI) mass spectra were obtained with a JMS-AX 505 HAD spectrometer (JEOL) at an ionization voltage of 70 eV. Electrospray ionization (ESI) mass spectra were measured with a PE SCIEX API III biomolecular mass analyzer.

Chromatography Column chromatography: Silica gel 60 (70—230 mesh, Merck), and ODS Cosmosil 140 C18-OPN (Nacalai Tesque, Kyoto, Japan). Medium pressure liquid chromatography (MPLC) was performed on a LiChroprep Si 60 column or LiChroprep RP-18 column (both size A, Merck, Darmstadt, Germany). Gas chromatography-mass spectra (GC-MS) were obtained using a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) fitted with a DB-1 column [0.25 mm (i.d)×30 m] (J & W Scientific, U.S.A.), coupled to an automass system II benchtop quadrupole mass spectrometer (JEOL) under the following conditions: column temperature, 50 °C for 10 min and gradient to 250 °C (10 °C/min) for 20 min; injection temperature, 250 °C or isothermal at 30 °C for 30 min, and 170 °C for methyl esters of short chain fatty acids; carrier gas, He (flow rate, 15 ml/min). Thin-layer chromatography (TLC) was performed with Silica gel 60 F_{254} and RP-18 F_{254} S plates, both 0.25 mm thickness (Merck, Darmstadt), and spots were detected under UV light or after spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

Chemicals and Enzymes Compounds **1**—**8** were obtained from the seeds of *Croton tiglium* as reported previously.[8] Rat brain protein kinase C (PKC, specific activity, 100 units/ml), staurosporine and L-α-phosphatidyl-L-serine were purchased from Sigma (St. Louis, U.S.A.). A protein kinase enzyme assay system (code RPN 77 kit) was purchased from Amersham International (Buckinghamshire, England). [γ-^{32}P]ATP was supplied at specific activity of 370 MBq/ml (10 mCi/ml) from Amersham. Benzamidine HCl was obtained from Tokyo Kasei Org. Chemicals (Tokyo, Japan). Ethylene diamine tetraacetic acid (EDTA), ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA), phenyl methylsulphonyl fluoride, β-mercaptoethanol, Tris/HCl and orthophosphoric acid were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fatty acid methyl esters were from Nacalai Tesque (Kyoto, Japan).

Cells The HTLV-I-carrying cell line MT-4 cells and the human leukemia T-cell line MOLT-4 cells were used. They were maintained at 37 °C under 5% CO_2 in RPMI-1640 medium (Flow Laboratories, Irvine, Scotland), supplemented with 10% fetal calf serum (FCS, Flow Laboratories, North Ryde, Australia), 100 μg/ml of streptomycin (Meiji Seika, Tokyo) and 100 U/ml of penicillin G (Banyu Pharmaceutical, Tokyo).

Virus The LAI strain of HIV-1 was obtained from a culture supernatant of MOLT-4 cells that had been persistently infected with the LAI strain.

Inhibition of CPE on MT-4 Cells The inhibition of HIV-1-induced CPE was measured by the method of Harada *et al.*[34] MT-4 cells were in-

fected for 1h with the LAI strain of HIV-1 at $TCID_{50}$ of 0.001/cell (determined by MT-4 cells on day 5 after infection), and the non-adsorbed virus was removed by washing. Then, the cells were re-suspended at 1.5×10^5 cells/ml in RPMI-1640 medium. Then, 200 μl/well of the cell suspension was cultured for 5 d in a 96-well culture plate containing various concentrations of a test compound. Control assays were performed without the test compounds in HIV-1-infected and -uninfected cultures. On day 5, the concentration of the test compound that completely prevented CPE (IC_{100}) and the concentration that reduced the viability of MT-4 cells (CC_0) were determined through an optical microscope.

Activation of Protein Kinase C The PKC activation was assayed by measuring the incorporation of ^{32}P radioactivity from [γ-^{32}P]ATP into a peptide, Arg-Lys-Arg-Thr-Leu-Arg-Arg-Leu-OH, using a Biotrak PKC enzyme assay system, except that TPA in the kit was replaced by the tested compounds (10 ng/ml) dissolved in DMSO (a final concentration of DMSO did not exceed 0.02%). The reaction mixture (55 μl) contained 2 milli units of PKC, 50 mM Tris/HCl (pH 7.5), 0.13% w/v mercaptoethanol, 2.1 mM EDTA, 4.2 mM EGTA, 20.9 μg/ml phenyl methyl sulphonyl fluoride, 4.2 mM benzamidine, 1.3 μM calcium acetate, 75 μM peptide, 34 μg/ml L-α-phosphatidyl-L-serine, 3.4 mM DTT, 0.68 μM sodium azide, and 6.5 nM $MgCl_2$. After the addition of 0.55 nM [γ-^{32}P]ATP (50×10^3 cpm/nmol), the reaction mixture was mixed and maintained at 37 °C for 30 min. Reactions were terminated by the addition of 10 μl of an ice-cold stop reagent. Aliquots of 35 μl were transferred to the center of peptide binding discs. After 10 min, the discs were washed two times with 75 mM orthophosphoric acid. The radioactivity of ^{32}P-labeled samples was counted in 10 ml of a scintillation fluid for 1 min. In the presence of TPA, lipid and Ca acetate, PKC activation represents 100% and corresponds to 8400 nmol/mg/min (positive control). Values for the activation of PKC by the tested compounds are given as the mean of duplicate determinations, and calculated relative to that of the positive control (TPA). Blank (in the absence of PKC) and control (in the absence of TPA or tested samples) tests were also carried out. One unit of PKC was defined as that amount of enzyme which incorporated 1 nmol of phosphate from ATP into its substrate, peptide, per min under the assay conditions described above. In the presence of 34 μg/ml L-α-phosphatidyl-L-serine, 1.3 μM Ca acetate and 2.7 μg/ml TPA, PKC activity was completely inhibited by staurosporine at a concentration of 180 nM.

Preparation of Phorbol (9), Isophorbol (14) and 4-Deoxy-4α-isophorbol (23) A hexane-soluble fraction (50 g) of the methanol extract of the seeds of *C. tiglium*, as reported previously,[8] was mixed with a solution of $Ba(OH)_2\cdot8H_2O$ (2.2% in MeOH, 500 ml) and stirred under an atmosphere of argon for 20 h at room temperature. The procedure was followed as reported previously[9,10] to obtain a crude phorbol fraction (10 g). Column chromatography of the fraction on SiO_2 using $CHCl_3$–MeOH (9.5 : 0.5—7 : 3) as an eluent and subsequent purification with MPLC (RP-18, MeOH–H_2O, 4 : 6) yielded phorbol (**9**, 537 mg),[11—13] isophorbol (**14**, 185 mg)[11—13] and 4-deoxy-4α-phorbol (**23**, 10 mg).[35,36]

Preparation of Phorbol 12-Acetate (9a), Phorbol 12,13-Diacetate (9c), Phorbol 12,13,20-Triacetate (9d), 4-*O*-Methylphorbol 12,13,20-Triacetate (9e), 3β-Hydroxyphorbol 12,13,20-Triacetate (9f), and Phorbol 12,13,20-Tribenzoate (10) Acetylation of **9** with Ac_2O/pyridine at 90 °C for 1 h gave phorbol 12,13,20-triacetate (**9d**).[16,17] Methylation of **9d** with CH_3I/Ag_2O in DMF at room temperature for 20 h gave 4-*O*-methylphorbol 12,13,20-triacetate (**9e**),[14] while the reduction of **9d** with $NaBH_4$ gave 3β-hydroxyphorbol 12,13,20-triacetate (**9f**).[13] When Ac_2O/*p*-TsOH was used, **9d** gave phorbol 4,12,13,20-tetraacetate (**9g**),[16] while the reaction of **9** with benzoyl chloride/pyridine gave phorbol 12,13,20-tribenzoate (**10**).[15,16] A solution of **9d** (50 mg) in 0.05 M KOH/MeOH (10 ml) was stirred at room temperature for 1 h to give phorbol 12-acetate (**9a**) (40 mg, 80%). Selective hydrolysis of **9d** (30 mg) with $HClO_4$ afforded phorbol 12,13-diacetate (**9c**, 24 mg, in a yield of 80%) [oil. EI-MS *m/z* 338 $[M-CH_3COOH]^+$, 328 $[M-2\times CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.0 and 2.12 (each 3H, s, $2\times C\underline{H}_3CO$–), 5.4 (1H, d, *J*=10.2, H-12) and 4.0 (2H, ABq, *J*=12.5, H_2-20)].

Preparation of Phorbol 13-Acetate (9b) Selective hydrolysis of **1** with $HClO_4$ afforded **9b**.[18]

Preparation of Isophorbol 12,13,20-Triacetate (14c), Isophorbol 12,13,20-Tributylate (14d), Isophorbol 4,12,13,20-Tetraacetate (14e), and a Photo Product (22) Acylation of **14** (36 mg) with butyryl chloride in pyridine afforded **14d** (18 mg, in a yield of 50%). Acetylation of **14** gave **14c**[35] and **14e**.[19] Irradiation of **14c** with UV light (254 nm/5 h) afforded **22**.[19]

Preparation of 1a Compound **1a** was obtained from **1** by treatment with mesyl chloride/pyridine at room temperature for 21 h.[20] The compound had the following physicochemical and spectroscopic properties: oil. $[\alpha]_D$ +3° (*c*=0.05, $CHCl_3$). IR ν_{max} cm^{-1}: 3450 (OH), 2960 and 2930 (C=C), 2850, 1730 (ester C=O), 1600 (α,β-unsaturated ketone). UV λ_{max} (log ε) nm: 243 (3.55). EI-MS *m/z* 650 $[M]^+$, 632 $[M-H_2O]^+$, 590 $[M-AcOH]^+$, 572 $[M-AcOH-H_2O]^+$, 370 [M−linoleic acid]$^+$, 352 [M−linoleic acid−H_2O]$^+$, 310 [M−linoleic acid−AcOH]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, m, C$\underline{H}_3$, linoloyl), 1.10 (3H, d, *J*=7.4 Hz, H_3-18), 1.32 (14H, br d, $7\times C\underline{H}_2$, linoloyl), 1.57 (3H, s, H_3-17), 1.60 (2H, m, CO–CH_2–$C\underline{H}_2$, linoloyl), 1.80 (3H, m, H_3-19), 2.08 (3H, s, $COC\underline{H}_3$), 2.10 (4H, m, $C\underline{H}_2$–CH=CH–CH_2–CH=CH–$C\underline{H}_2$, linoloyl), 2.35 (2H, m, CO–$C\underline{H}_2$, linoloyl), 2.38 (1H, d, *J*=19.0 Hz, H_b-5), 2.57 (1H, d, *J*=19.0 Hz, H_a-5), 2.76 (2H, dd, *J*=13 and 3.8 Hz, =CH–$C\underline{H}_2$–CH=, linoloyl), 3.1 (1H, t, *J*=2.2 Hz, H-10), 3.25 (1H, m, H-11), 3.40 (1H, d, *J*=8.8 Hz, H-14), 3.50 (1H, dd, *J*=8.8 and 5.5 Hz, H-8), 4.44 (2H, ABq, *J*=15.0 Hz, H_2-20), 4.90 (1H, s, H_a-16), 5.00 (1H, s, H_b-16), 5.10 (1H, t, *J*=2.1 Hz, H-12), 5.34 (4H, m, –($C\underline{H}$=$C\underline{H}$)$_2$, linoloyl), 5.50 (1H, d, *J*=5.5 Hz, H-7), and 7.60 (1H, t, *J*=2.2 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ: 11.0 (C-19), 14.9 ($\underline{C}H_3$, linoloyl), 17.3 (C-18), 18.6 (C-17), 21.4 ($CO\underline{C}H_3$), 23.6 ($\underline{C}H_2$–CH_3, linoloyl), 26.1 (CO–CH_2–$\underline{C}H_2$, linoloyl), 26.7 (CH=CH–$\underline{C}H_2$–CH=CH–, linoloyl), 28.3 ($\underline{C}H_2$–CH=CH–CH_2–CH=CH–$\underline{C}H_2$, linoloyl), 30.3—30.8 ($4\times\underline{C}H_2$, linoloyl), 33.1 ($\underline{C}H_2$, linoloyl), 32.7 ($\underline{C}H_2$, linoloyl), 35.4 (CO–$\underline{C}H_2$, linoloyl), 37.8 (C-11), 44.1 (C-8), 50.0 (C-14), 57.2 (C-10), 70.7 (C-20), 78.6 (C-4), 86.5 (C-9), 117.4 (C-16), 121.3 (C-12), 129.1 and 129.2 ($\underline{C}H$=$\underline{C}H$, linoloyl), 130.0 (C-7), 131.1 and 131.2 ($\underline{C}H$=$\underline{C}H$, linoloyl), 134.6 (C-2), 136.5 (C-6), 144.1 (C-13), 148.0 (C-15), 160.1 (C-1), 172.1 (C=O, linoloyl), 175.5 (C=O, $\underline{C}OCH_3$), and 215.5 (C-3).

Preparation of 12-*O*-,13-*O*-Diacetylphorbol 20-(9*Z*,12*Z*-Tetradecadienoate) (1b), 12-*O*-Decanoyl-13-*O*-(2-methylbutyryl)phorbol 20-Acetate (4a), 12-*O*-,20-*O*-Diacetylphorbol 13-Decanoate (6a) and 12-*O*-Tetradecanoylphorbol 13,20-Diacetate (8a) The respective acetylation of **1**, **4**, **6**, and **8** gave **1b** (85% in yield), **4a** (90%), **6a** (85%), and **8a** (70%), respectively. These compounds had the following properties: **1b**: oil. EI-MS *m/z*, 710 $[M]^+$, 650 $[M-CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.0—212 (6H, s, $2\times C\underline{H}_3CO$–) and 5.4 (1H, d, *J*=10.2 Hz, H-12); **4a**: oil. EI-MS *m/z* 644 $[M]^+$, 584 $[M-CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.1 (3H, s, $C\underline{H}_3CO$–) and 4.42 (2H, ABq, *J*=13.5 Hz, H_2-20); **6a**: oil. EI-MS *m/z* 602 $[M]^+$, 542 $[M-CH_3COOH]^+$, 482 $[M-2\times CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.04 and 2.1 (each 3H, s, $C\underline{H}_3CO$–) and 4.43 (2H, ABq, *J*=15.0 Hz, H_2-20); **8a**: oil. EI-MS *m/z* 658 $[M]^+$, 598 $[M-CH_3COOH]^+$, 538 $[M-2\times CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06—2.10 (each 3H, s, $C\underline{H}_3CO$–) and 4.42 (2H, ABq, *J*=12.5 Hz, H_2-20).

Preparation of 12-*O*-,20-*O*-Diacetyl-4-*O*-methylphorbol 13-Decanoate (6b) and 4-*O*-Methyl-12-*O*-tetradecanoylphorbol 13,20-Diacetate (8b) Methylation of **6** and **8** with MeI and Ag_2O gave **6b** (6 mg, 30%) and **8b** (5 mg, 25%), respectively. These compounds had the following properties. **6b**: oil. EI-MS *m/z* 616 $[M]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.08 and 2.13 (each 3H, s, $C\underline{H}_3CO$–), and 3.27 (3H, s, $C\underline{H}_3O$–); **8b**: oil. EI-MS *m/z* 672 $[M]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 and 2.1 (each 3H, s, $C\underline{H}_3CO$–) and 3.27 (3H, s, $C\underline{H}_3O$–).

Preparation of 12-*O*-Acetylphorbol 13,20-Ditetradecanoate (8d), 12-*O*-Acetylphorbol 13,20-Dihexanoate (11a), 12-*O*-Acetylphorbol 13,20-Dinonanoate (12a) and 12-*O*-Acetylphorbol 13,20-Didodecanoate (13a) A pyridine solution (1.5 ml) containing 2 mM acyl chloride and 0.12 mM of **9a** (50 mg) was stirred at 0 °C under an atmosphere of argon, and then at room temperature for 5 d, with products monitored by TLC, to give the respective 12-*O*-acetyl-13-*O*-,20-*O*-diacyl derivatives in yields of 60—80%. **8d**: oil (30 mg, 60% from **9a**). EI-MS *m/z* 826 $[M]^+$, 766 $[M-CH_3COOH]^+$, 598 $[M-CH_3(CH_2)_{12}COOH]^+$, 370 $[M-2\times CH_3-(CH_2)_{12}-COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, $C\underline{H}_3CO$–), 5.45 (1H, d, *J*=10.5 Hz, H-12), 4.45 (2H, ABq, *J*=12.0 Hz, H_2-20), and signals for two tetradecanoyl moieties at δ 2.3 (4H, $2\times C\underline{H}_2$), 1.60 (4H, $2\times C\underline{H}_2$), 1.2 (40H, $2\times10C\underline{H}_2$) and 0.09 (6H, $2\times C\underline{H}_3$); **11a**: oil (32.5 mg, 65% from **9a**). EI-MS *m/z* 602 $[M]^+$, 542 $[M-CH_3COOH]^+$, 486 $[M-CH_3(CH_2)_4COOH]^+$, 370 $[M-2\times CH_3(CH_2)_4COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, $C\underline{H}_3CO$–), 5.45 (1H, d, *J*=10.0 Hz, H-12), 4.43 (2H, ABq, *J*=12.0 Hz, H_2-20), and signals for two hexanoyl moieties at 2.3 (4H, $2\times C\underline{H}_2$), 1.60 (4H, $2\times C\underline{H}_2$), 1.2 (12H, $6\times C\underline{H}_2$) and 0.09 (6H, $2\times C\underline{H}_3$); **12a**: oil (40 mg, 80% from **9a**). EI-MS *m/z* 626 $[M]^+$, 528 $[M-CH_3(C\underline{H}_2)_7COOH]^+$, 370 $[M-2\times CH_3(CH_2)_7COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s), 5.45 (1H, d, *J*=10.0 Hz, H-12), 4.41 (2H, ABq, *J*=12.0 Hz, H_2-20), and signals for two octanoyl moieties at 2.30 (4H, $2\times C\underline{H}_2$), 1.62 (4H, $2\times C\underline{H}_2$), 1.2 (20H, $10\times C\underline{H}_2$) and 0.09 (6H, $2\times C\underline{H}_3$); **13a**: oil (37.5 mg, 75% from **9a**). EI-MS *m/z* 770 $[M]^+$, 570 $[M-CH_3(CH_2)_{10}COOH]^+$, 510 $[M-CH_3COOH-CH_3(CH_2)_{10}COOH]^+$, 370 $[M-2\times CH_3(CH_2)_{10}COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s), 5.45 (1H,

d, J=10.0 Hz, H-12), 4.45 (2H, ABq, J=14.0 Hz, H_2-20), and signals of two dodecanoyl moieties at 2.30 (4H, 2×C$\underline{H}_2$), 1.60 (2H, C$\underline{H}_2$), 1.20 (32H, 16×C$\underline{H}_2$) and 0.09 (6H, 2×C$\underline{H}_3$).

Preparation of 12-*O*-Acetylphorbol 13-Tetradecanoate (8c), 12-*O*-Acetylphorbol 13-Hexanoate (11), 12-*O*-Acetylphorbol 13-Octanoate (12) and 12-*O*-Acetylphorbol 13-Dodecanoate (13) by Partial Hydrolysis The 12-*O*-acetyl-13-*O*-,20-*O*-diacylphorbol derivatives obtained (**8d**, **11a**, **12a** and **13a**) were separately hydrolyzed (each 30 mg) with 70% $HClO_4$/MeOH to give the respective 12-*O*-acetyl-13-*O*-acylphorbol derivatives (**8c**, **11**, **12** and **13**) in yields of 60—70%. All products were purified by column chromatography on SiO_2, followed by MPLC (RP-18). The structures of these compounds were established by spectroscopic methods and their characteristics are described below. **8c**: oil (21 mg, 70% from **8d**). EI-MS m/z 616 $[M]^+$, 556 $[M-CH_3COOH]^+$, 388 $[M-CH_3(CH_2)_{12}COOH]^+$, 370 $[M-H_2O-CH_3(CH_2)_{12}COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, C$\underline{H}_3$CO–), 5.45 (1H, d, J=10.0 Hz, H-12), 4.00 (2H, ABq, J=12.0 Hz, H_2-20), and signals for tetradecanoyl moiety at δ: 2.3 (2H, C$\underline{H}_2$), 1.62 (2H, C$\underline{H}_2$), 1.2 (20H, 10×C$\underline{H}_2$) and 0.09 (3H, C$\underline{H}_3$); **11**: oil (18 mg, 60% from **11a**). EI-MS m/z 504 $[M]^+$, 444 $[M-CH_3COOH]^+$, 388 $[M-CH_3(CH_2)_4COOH]^+$, 370 $[M-H_2O-CH_3(CH_2)_4COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, C$\underline{H}_3$CO–), 5.45 (1H, d, J=10.0 Hz, H-12), 4.00 (2H, ABq, J=12.0 Hz, H_2-20), and signals for hexanoyl moiety at 2.3 (2H, C$\underline{H}_2$), 1.62 (2H, C$\underline{H}_2$), 1.2 (4H, 2×CH_2) and 0.09 (3H, C$\underline{H}_3$); **12**: oil (21 mg, 70% from **12a**). EI-MS m/z 546 $[M]^+$, 486 $[M-CH_3COOH]^+$, 388 $[M-CH_3(CH_2)_7COOH]^+$, 370 $[M-H_2O-CH_3(CH_2)_7COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, CH_3–CO–), 5.45 (1H, d, J=10.0 Hz, H-12), 4.00 (2H, ABq, J=12.0 Hz, H_2-20), and signals for octanoyl moiety at 2.30 (2H, C$\underline{H}_2$), 1.61 (2H, C$\underline{H}_2$), 1.2 (10H, 5×C$\underline{H}_2$) and 0.09 (3H, C$\underline{H}_3$); **13**: oil (19.5 mg, 65% from **13a**). EI-MS m/z 570 $[M]^+$, 510 $[M-CH_3COOH]^+$, 388 $[M-CH_3(CH_2)_{10}COOH]^+$, 370 $[M-H_2O-CH_3(CH_2)_{10}COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s), 5.45 (1H, d, J=10.0 Hz, H-12), 4.00 (2H, ABq, J=12.0 Hz, H_2-20), and signals for dodecanoyl moiety at 2.3 (2H, C$\underline{H}_2$), 1.62 (2H, C$\underline{H}_2$), 1.20 (16 H, 8×C$\underline{H}_2$) and 0.09 (3H, C$\underline{H}_3$).

Preparation of 13-*O*-Acetylisophorbol (14a) and 13-*O*-,20-*O*-Diacetylisophorbol (14b) Acetic anhydride (50 μl) was added to an ice cooled solution of isophorbol (**14**, 50 mg, 0.14 mmol) in pyridine (1 ml), and the mixture was stirred at 60 °C for 1 h. The reaction mixture was worked up as usual to afford **14a** (8 mg, 14%) and **14b** (22 mg 36%). **14a**: oil. EI-MS m/z 406 $[M]^+$, 388 $[M-H_2O]^+$, 370 $[M-2H_2O]^+$, 352 $[M-3H_2O]^+$, 309 $[M-3H_2O-CH_3CO-]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s, CO$\underline{CH}_3$); **14b**: oil. EI-MS m/z 448 $[M]^+$, 430 $[M-H_2O]^+$, 412 $[M-2H_2O]^+$, 369 $[M-2H_2O-CH_3CO-]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.11, 2.09 (6H, 2s, CO$\underline{CH}_3$), 4.33 (2H, ABq, J=12.4 Hz, H-20).

Preparation of 12-*O*-Octanoylisophorbol 13,20-Diacetate (15), 12-*O*-Decanoylisophorbol 13,20-Diacetate (16), 12-*O*-(10-Undecenoyl)isophorbol 13,20-Diacetate (17), 12-*O*-Dodecanoylisophorbol 13,20-Diacetate (18), 12-*O*-Tetradecanoylisophorbol 13,20-Diacetate (19), 12-*O*-Heptadecanoylisophorbol 13,20-Diacetate (20) and 12-*O*-(1-Adamantanoyl)-isophorbol 13,20-Diacetate (21) Acyl chloride (400 μl, 0.44 mmol) was added to an ice cooled solution of **14a** (50 mg, 0.11 mmol) in pyridine (1 ml), and the mixture was stirred at room temperature for 1—3 d. The reaction mixture was worked up as usual to give the respective products in yields of 43—74%. **15**: oil (45 mg, 70%). EI-MS m/z 574 $[M]^+$, 556 $[M-H_2O]^+$, 514 $[M-H_2O-CH_3CO-]^+$, 496 $[M-2H_2O-CH_3CO-]^+$, 429 $[M-H_2O-C_8H_{15}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.10, 2.07 (6H, 2s, CO$\underline{CH}_3$), 2.33 (2H, m, CH_2), 4.33 (2H, ABq, J=12.2 Hz, H-20), 5.47 (1H, d, J=10.2 Hz, H-12), and signals for octanoyl moiety at δ: 0.87 (3H, t, J=6.6 Hz, CH_3), 1.31 (8H, m, 4×CH_2), 1.68 (2H, m, CH_2); **16**: oil (43 mg, 64%). EI-MS m/z 602 $[M]^+$, 583 $[M-H_2O]^+$, 524 $[M-H_2O-CH_3COOH]^+$, 481 $[M-H_2O-CH_3COOH-CH_3COO-]^+$, 429 $[M-H_2O-C_{10}H_{19}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09, 2.07 (6H, 2s, CO$\underline{CH}_3$), 2.35 (2H, m, CH_2), 4.33 (2H, ABq, J=12.5 Hz, H-20), 5.47 (1H, d, J=10.3 Hz, H-12), and signals for decanoyl moiety at δ: 0.88 (3H, t, J=6.6 Hz, CH_3), 1.27 (15H, m, H-16, 6×CH_2), and 1.67 (2H, m, CH_2); **17**: oil (51 mg, 74%). EI-MS m/z 614 $[M]^+$, 596 $[M-H_2O]^+$, 536 $[M-H_2O-CH_3COOH]^+$, 429 $[M-H_2O-$(10-undecenoyl)$]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09, 2.06 (6H, 2s, CO$\underline{CH}_3$), 4.34 (2H, ABq, J=12.4 Hz, H-20), 5.47 (1H, d, J=10.3 Hz, H-12), and signals for 10-undecenoyl moiety at δ: 1.3—1.6 [(2H, m, CH_2=CH–(C$\underline{H}_2$)$_7$–CH_2–), 2.03 [(2H, m, CH_2=CH–(C$\underline{H}_2$)$_7$–CH_2–), 2.6 [(2H, t, J=8.04 Hz, CH_2=CH–(CH_2)$_7$–C$\underline{H}_2$–), 4.94 [(2H, m, C$\underline{H}_2$=CH–(CH_2)$_8$–), and 5.81 [(1H, m, CH_2=C$\underline{H}$–(CH_2)$_8$–); **18**: oil (47 mg, 67%). EI-MS m/z 630 $[M]^+$, 612 $[M-H_2O]^+$, 534 $[M-2H_2O-CH_3COOH]^+$, 475 $[M-2H_2O-CH_3COOH-CH_3COO-]^+$, 429 $[M-H_2O-C_{12}H_{23}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.10, 2.08 (6H, 2s, CO$\underline{CH}_3$), 4.32 (2H, ABq, J=12.5 Hz, H-20), 5.46 (1H, d, J=10.3 Hz, H-12), and signals for dodecanoyl moiety at δ: 0.87 (3H, t, J=6.6 Hz, CH_3), 1.27 (19H, m, H-16, 8×CH_2), 1.67 (2H, m, CH_2), and 2.36 (2H, m, CH_2); **19**: oil (42 mg, 57%). EI-MS m/z 658 $[M]^+$, 640 $[M-H_2O]^+$, 597 $[M-H_2O-CH_3CO-]^+$, 538 $[M-H_2O-CH_3COO-CH_3CO-]^+$, 429 $[M-H_2O-C_{14}H_{27}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09, 2.07 (6H, 2s, CO$\underline{CH}_3$), 4.33 (2H, ABq, J=12.4 Hz, H-20), 5.47 (1H, d, J=10.3 Hz, H-12), and signals for tetradecanoyl moiety at δ: 0.88 (3H, t, J=6.8 Hz, CH_3), 1.26 (23H, m, H-16, 9×CH_2), 1.64 (2H, m, CH_2), and 2.32 (2H, m, CH_2); **20**: Oil (50 mg, 64%). EI-MS m/z 700 $[M]^+$, 682 $[M-H_2O]^+$, 587 $[M-H_2O-CH_3COO-]^+$, 569 $[M-2H_2O-CH_3COO-]^+$, 429 $[M-H_2O-C_{17}H_{33}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09, 2.07 (6H, 2s, CO$\underline{CH}_3$), 4.33 (2H, ABq, J=12.4 Hz, H-20), 5.47 (1H, d, J=10.3 Hz, H-12), and signals for heptadecanoyl moiety at δ: 0.88 (3H, t, J=6.8 Hz, CH_3), 1.25 (29H, m, H-16, 13×CH_2), 1.63 (2H, m, CH_2), and 2.31 (2H, m, CH_2); **21**: oil (45 mg, 66%). EI-MS m/z 610 $[M]^+$, 592 $[M-H_2O]^+$, 532 $[M-H_2O-CH_3COOH]^+$, 429 $[M-H_2O$-1-(1-adamantnoyl)$]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09, 2.07 (6H, 2s, CO$\underline{CH}_3$), 4.33 (2H, ABq, J=12.0 Hz, H-20), 5.44 (1H, d, J=10.5 Hz, H-12), and signals for 1-adamantanoyl moiety at δ: 1.73 (7H, m), 1.91 (6H, m, H-11), and 2.06 (3H, m).

Preparation of 13-*O*-Acetyl(4-deoxy-4α-phorbol) (23a), 13-*O*-,20-*O*-Diacetyl(4-deoxy-4α-phorbol) (23b) and 12-*O*-,13-*O*-,20-*O*-Triacetyl(4-deoxy-4α-phorbol) (23c) Acetylation of **23** with Ac_2O/pyridine at 60 °C for 1 h afforded **23a** (21%), **23b** (35%) and **23c** (11%). **23a**: oil. EI-MS m/z 390 $[M]^+$, 372 $[M-H_2O]^+$, 354 $[M-2H_2O]^+$, 329 $[M-H_2O-CH_3CO-]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.09 (3H, s, CO$\underline{CH}_3$); **23b**: oil. EI-MS m/z 432 $[M]^+$, 414 $[M-H_2O]^+$, 354 $[M-H_2O-CH_3COOH]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.11 (6H, 2s, CO$\underline{CH}_3$), and 4.47 (2H, ABq, J=11.8 Hz, H-20); **23c**[36]: oil. EI-MS m/z 474 $[M]^+$, 431 $[M-CH_3CO-]^+$, 413 $[M-H_2O-CH_3CO-]^+$, 353 $[M-H_2O-CH_3COOH-CH_3CO-]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.11, 2.07 (9H, 3s, CO$\underline{CH}_3$), 4.40 (2H, ABq, J=12.7 Hz, H-20), and 5.44 (1H, d, J=10.5 Hz, H-12).

Preparation of 12-*O*-Decanoyl(4-deoxy-4α-phorbol) 13,20-Diacetate (24) and 12-*O*-Tetradecanoyl(4-deoxy-4α-phorbol) 13,20-Diacetate (25) Acyl chloride (400 μl, 0.48 mmol) was added to an ice cooled solution of **23b** (50 mg, 0.12 mmol) in pyridine (1 ml), and the mixture was stirred at room temperature for 24 h. The reaction mixture was worked up as usual to give **24** (71%) and **25** (68%). **24**: oil. EI-MS m/z 586 $[M]^+$, 543 $[M-CH_3CO-]^+$, 526 $[M-CH_3COOH]^+$, 413 $[M-H_2O-C_{10}H_{19}O]^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.06 (6H, 2s, CO$\underline{CH}_3$), 4.40 (2H, ABq, J=12.5 Hz, H-20), 5.47 (1H, d, J=10.5 Hz, H-12), and signals for decanoyl moiety at δ: 0.88 (3H, t, J=7.1 Hz, CH_3), 1.28 (12H, m, 6×CH_2), 2.36 (2H, m, CH_2), and 2.59 (2H, t, J=7.8 Hz, CH_2); **25**: oil. EI-MS m/z 642 $[M]^+$, 599 $[M-CH_3CO-]^+$, 582 $[M-CH_3COOH]^+$, 522 $[M-2CH_3COOH]^+$, 413 $[M-H_2O-C_{14}H_{27}O]$. ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.06 (6H, 2s, CO$\underline{CH}_3$), 4.14 (2H, ABq, J=12.5 Hz, H-20), 5.47 (1H, d, J=10.8 Hz, H-12), and signals for tetradecanoyl moiety at δ: 0.88 (3H, t, J=7.1 Hz, CH_3), 1.27 (16H, m, 6×CH_2), 2.36 (2H, m, CH_2), and 2.59 (2H, t, J=7.8 Hz, CH_2).

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