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A New Class of Simplified Phorbol Ester Analogues: Synthesis and Binding to PKC and ηPKC-C1B (ηPKC-CRD2)

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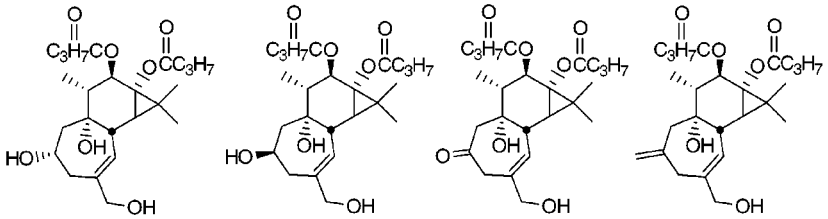
ABSTRACT



A unique class of simplified phorbol ester analogues is described for the first time. A highly efficient retro-annelation sequence was developed in order to remove the five-membered ring from the phorbol diterpene core, allowing access to BCD ring analogues of the phorbol esters. The binding of these analogues to protein kinase C (PKC) and the truncated peptide ηPKC-C1B (ηPKC-CRD2) is also reported.

As the most potent tumor promoters known, the phorbol esters have played a unique role in the discovery of multistage carcinogenesis and continue to be central to its further elucidation including efforts to develop prevention strategies.[1] More recently, these plant-derived compounds and their derivatives have also served as effective molecular probes in numerous biochemical and physiological processes, ranging from signal transduction, HIV expression, and antigen presentation for monoclonal antibody drug delivery to molecular aspects of learning.[2] The activities of the phorbol esters are proposed to arise from their binding to the regulatory domain of protein kinase C (PKC).[3] This site is normally reserved for the binding of the endogenous PKC activators, diacyl glycerols, but it is also implicated as the recognition domain of the therapeutically promising bryostatins.[4]

Numerous ester derivatives of phorbol have been synthesized in efforts to define the molecular basis for the activities of the phorbol esters and to obtain isozyme-selective PKC activators.[5] In contrast, relatively little is known about modifications of the phorbol core system, a situation influenced in part by the difficulty of total synthesis in this series and problems in degradation arising from the sensitiv-

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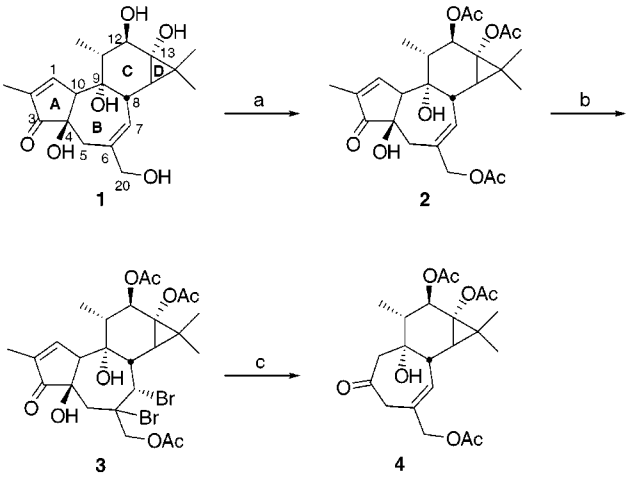
ity of phorbol to acid, base, heat, and light and its hazardous nature.[6,7] We describe herein the synthesis of the first members of a new and simplified class of phorbol ester skeletal analogues and their binding to PKC and η-C1B, a surrogate of the regulatory domain of η-PKC.[8]

We previously reported an analysis of all pharmacophoric triads of phorbol. The best correlation between atom type/position with activity was found with the oxygens at C4, C9, and C20. The C3 oxygen was noted as a possible surrogate of the C4 oxygen.[9] To obtain further information on the role of these oxygen atoms in the binding and the activities of the phorbol esters, we have now developed a procedure for the concise removal of the A ring from readily available phorbol derivatives. This procedure entails initial conversion of phorbol (**1**) to its triacetate **2** and treatment of the latter with NBS/NaOAc in aqueous DME [Scheme 1] in order to protect the B ring double bond as the dibromide.[10] Ozonolysis of the resultant dibromide is followed by in situ treatment of the intermediate ozonide with Zn/HOAc/NaOAc, which serves both to reduce the ozonide and to cleave the dibromide, delivering ketone **4** in 48% yield.[11] The efficiency and brevity of this remarkably facile retro-annelation make it a highly useful strategy for accessing a wide range of novel phorbol ester analogues of biochemical, synthetic, and medicinal interest.

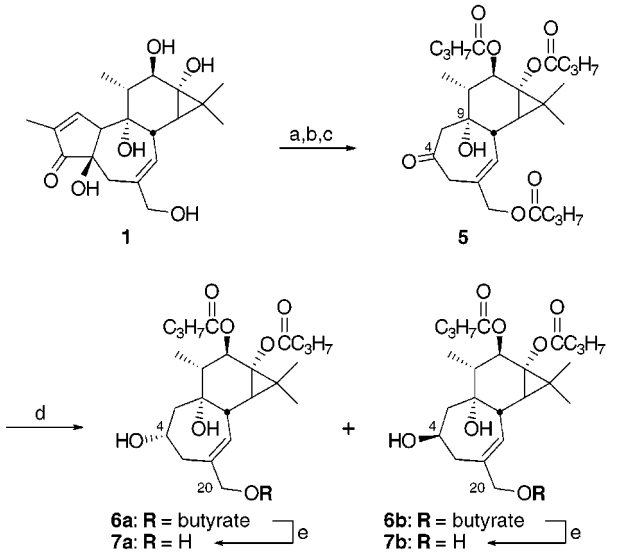
Scheme 1[a]



[a] (a) Ac_2O, pyridine, rt, 18 h, 90%; (b) NBS/NaOAc, DME/H_2O, rt, 20 h, 70%; (c) O_3, CH_2Cl_2, −78 °C → rt, then, Zn/NaOAc/HOAc, rt, 1 h, 48%.

While the above degradative sequence was developed initially with the acetate derivatives, the C12 and C13 dibutyrates were selected for affinity and functional assays.[12] Tributyrate **5** was thus prepared in a fashion analogous to that of **4**. Reduction of ketone **5** with $NaBH_4$ (MeOH, 0 °C) provided two epimeric C4 alcohols (1:1), which were separated chromatographically. Since reduction of **5** with $NaBH(OAc)_3$ provided only one of these epimers, resulting presumably from coordination to the C9 alcohol and delivery of hydride from the α-face of the carbonyl, this alcohol is assigned as the C4-βOH isomer.[13] Selective hydrolysis ($HClO_4$/ MeOH, rt, 48 h) of the C20 butyrate of each epimer afforded the epimeric triols **7a** and **7b**, respectively [Scheme 2].

Scheme 2[a]



[a] (a) Butyric anhydride, pyridine, rt, 18 h, 82%; (b) NBS/NaOAc, DME/H_2O, rt, 20 h, 78%; (c) O_3, CH_2Cl_2, −78 °C → rt, then, Zn/NaOAc/HOAc, rt, 1 h, 45%; (d) $NaBH_4$, MeOH, 0 °C, 30 min, then, HCl_{aq}, 5 min, **6a**, 37%; **6b**, 37%; (e) $HClO_4$, MeOH, rt, 48 h, **7a**, 67%; **7b**, 75%.

Attempts to prepare the C4 carbonyl analogue [compound **11**, Scheme 3] by deprotection of the C20 alcohol in **5** under

(5) For examples of structure−activity studies, see: (a) Kazanietz, M. G.; Araces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. *Mol. Pharmacol.* **1993**, *44*, 298−307. (b) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917−924. (c) Irie, K.; Ishii, T.; Ohigashi, H.; Wender, P. A.; Miller, B. L.; Takeda, N. *J. Org. Chem.* **1996**, *61*, 2164−2173. (d) Sodeoka, M.; Arai, M. A.; Adachi, K.; Uotsu, K.; Shibasaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 457−458. (e) Winkler, J. D.; Kim, S.; Harrison, S.; Lewin, N. E.; Blumberg, P. M. *J. Am. Chem. Soc.* **1999**, *121*, 296−300.

(6) (a) Wender, P. A.; Lee, H. Y.; Wilhelm, R. S.; Williams, P. D. *J. Am. Chem. Soc.* **1989**, *111*, 8954−8957. (b) Wender, P. A.; Kogen, H.; Lee, H. Y.; Munger J. D. Jr.; Wilhelm, R. S.; Williams, P. D. *J. Am. Chem. Soc.* **1989**, 8957−8958. (c) Wender, P. A.; McDonald, F. E. *J. Am. Chem. Soc.* **1990**, *112*, 4956−4958. (d) Wender, P. A.; Rice, K. D.; Schnute, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 7897−7898.

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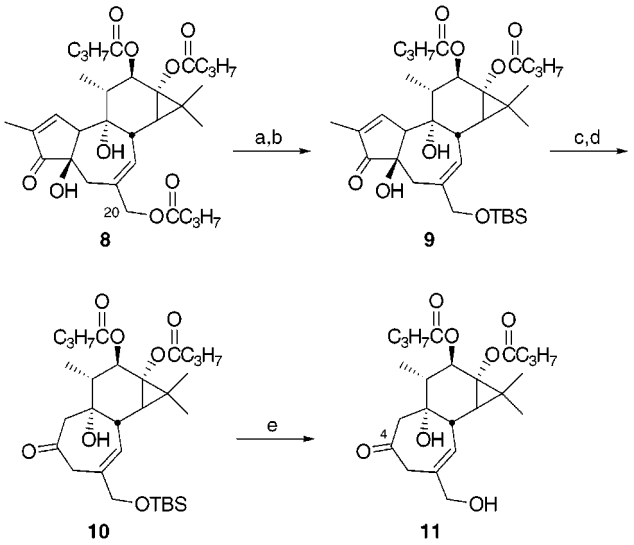
Scheme 3[a]



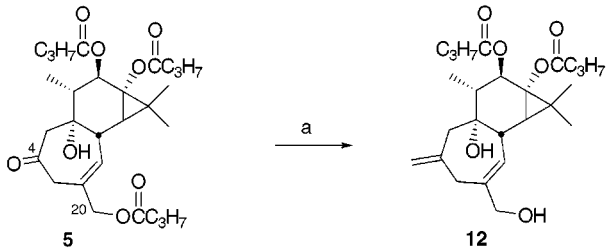
[a] (a) $HClO_4$, MeOH, rt, 48 h, 68%; (b) TBS-OTf/2,6-lutidine, CH_2Cl_2, 0 °C → rt, 15 min, 94%; (c) NBS/NaOAc, DME/H_2O, rt, 20 h, 74%; (d) O_3, CH_2Cl_2, −78 °C → rt, then, Zn/NaOAc/HOAc, rt, 1 h, 39%; (e) HF·pyridine (1:1), THF, rt, 5 h, 100%.

either mild acidic ($HClO_4$/MeOH) or mild basic ($Ba(OH)_2$/MeOH) conditions led to decomposition of the material. Therefore, an alternative route to the analogue **11** was designed.

Since selective C20 deprotection in phorbol esters is uneventful, it was decided that the C20 protecting group could be exchanged at the phorbol triester stage for a protecting group removable under neutral conditions.[14] Thus, selective hydrolysis of the C20 butyrate in **8** [Scheme 3] and subsequent reaction with TBS-OTf/2,6-lutidine (CH_2Cl_2, 0 °C → rt) afforded the desired C12,C13-dibutyryl C20-(*tert*-butyldimethylsilyloxy) phorbol derivative **9**. Temporary protection of the B ring double bond, ozonolysis, and global reduction gave ketone **10**, which was easily deprotected in quantitative yield using HF·pyridine (1:1) in THF to give ketone dibutyrate **11**.

In addition to derivatives **7a**, **7b**, and **11**, an analogue unable to participate in any hydrogen bond donor/acceptor interactions was targeted. The lack of a stereocenter at C4 in **12** along with its comparable conformational preferences made the exocyclic methylene derivative **12** an attractive candidate. The synthesis of **12** was achieved using the Nozaki−Takai protocol for the olefination of enolizable ketones.[15] The reaction led to the concurrent deprotection of C20 [Scheme 4]. Attempts to achieve this transformation

Scheme 4[a]



[a] (a) $TiCl_4/Zn/CH_2I_2$, THF, rt, 120 min, 31%.

under Wittig reaction conditions led to the reisolation of starting material. This observation is in line with the presumed propensity for enolization at the carbonyl center under basic reaction conditions.

Epimeric alcohols **7a** and **7b**, ketone **11**, and methylene derivative **12** were evaluated by competitive binding to a mixture of PKC isozymes (rat brain) and the second cysteine-rich domain of mouse skin PKCη (η-C1B). The results are summarized in Table 1.

Table 1. Affinities (in μM) of **7a**, **7b**, **11**, and **12** for PKC Isozyme Mix (Rat Brain) and η-C1B (Synthetic)[a]

	PKC		η-C1B	
compd	IC_{50}	K_i	IC_{50}	K_i
7a	>228	na[b]	>228	na
7b	>228	na	>228	na
11	2.1	0.61	3.6	0.26
12	49.5	20.6	22.5	4.2

[a] IC_{50} values were determined by nonlinear regression analysis from data obtained from competitive binding assays with ^{3}H-PDBu (based on the rapid filtration procedure of Tanaka[16]). For each IC_{50} determination, two independent experiments were performed, with each data point collected in triplicate. K_i values were calculated from IC_{50} values using the equation of Cheng and Prusoff.[17] K_d values for ^{3}H-PDBu were 4.1 and 2.3 nM for PKC-mix and η-C1B, respectively. [b] na = not available.

Both alcohols **7a** and **7b** were found to be only weak binders. However, the ketone **11** and derivative **12** showed

(8) (a) Irie, K.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Miller, B. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 353−356. (b) Yanai, Y.; Irie, K.; Ohigashi, H.; Wender, P. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 117−122. (c) Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U. *J. Am. Chem. Soc.* **1998**, *120*, 9159−9167. See also ref 4b.

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(10) The use of NaOAc was found to be essential for efficient dibromide formation. Unambiguous stereochemical assignment of C6 by NMR spectroscopy was not possible.

(11) All new compounds were fully characterized spectroscopically. The identity of the dibromide was further proven by elemental analysis.

(12) Driedger, P. E.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 567−571.

(13) (a) Phorbol numbering is used for all derivatives. (b) The ability of the C9 hydroxyl to act as a coordinating/activating group in a related stereoselective ketone reduction has been utilized in the total synthesis of phorbol: see ref 6b.

(14) See ref 1b.

(15) Hibino, J.; Okazoe, T.; Takai, K.; Nozaki, H. *Tetrahedron Lett.* **1985**, *26*, 5579−5580.

(16) Tanaka, Y.; Miyake, R.; Kikkawa, U.; Nishizuka, Y. *J. Biochem.* **1986**, *99*, 257−261.

significant affinities for both PKC and η-C1B. The difference between affinity to the isozyme mixture and to the pure surrogate is likely to be a simple consequence of the PKC mixture, isolated from rat brain, being largely composed of the α, β, and γ-PKC isozymes.

The difference in the binding affinities found for alcohols **7a** and **7b** and ketone **11** could be related to the hydrogen donor/acceptor properties of the oxygen functionality at C4. Alternatively, the relatively strong binding affinity achieved by ketone **11** and the exocyclic methylene derivative **12** might be a function of a conformational bias resulting from the influence of the sp-2 center at C4, suggesting that entropic restrictions could play a significant role. The *trans*-fusion between the A and B rings of the phorbol esters serves this function by rigidifying these molecules.

Further studies on the interplay of conformational restriction introduced by a C4 sp-2 center, the influence of changes in donor−acceptor properties, and the synthesis of novel analogues made possible through this new procedure are in progress.

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Supporting Information Available: Experimental details and full characterization of compounds **7a**, **7b**, **11**, and **12** (^{1}H NMR and ^{13}C NMR spectroscopy, IR spectroscopy, mass spectrometry, high-resolution mass spectrometry, and/or combustion analysis). This material is available free of charge via the Internet at http://pubs.acs.org.

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