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SYNTHESIS OF INGENOL ANALOGS WITH AFFINITY FOR PROTEIN KINASE C

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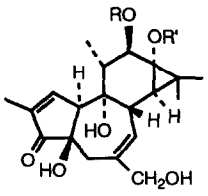
Abstract. The synthesis and preliminary evaluation of the first biologically active analogs of ingenol are described. Both saturated and unsaturated compounds containing both the C-3 and C-20 oxygen functionalities of ingenol have substantial affinity for protein kinase C.

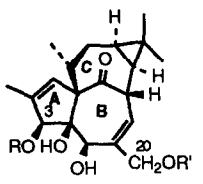
Protein kinase C is the phosphorylating enzyme mediating cellular signal transduction for a large class of hormones and cellular effectors that activate phosphatidylinositol 4,5-bis(phosphate) turnover.[3] Several structurally diverse naturally occurring compounds, including the bryostatins, teleocidin, asplysiatoxin, and esters of phorbol, **1**, and ingenol, **3** (Scheme I), mimic the function of diacyl glycerol, the endogenous activator of protein kinase C, but possess much greater potency. As a result, the study of the structural requirements for the activation of protein kinase C has focused on the synthesis and study of specifically modified derivatives of these natural product leads. Unlike phorbol, the structurally related diterpene ingenol has not yet yielded to total synthesis.[4,5]

One of the more imposing challenges in the synthesis of the highly oxygenated diterpene ingenol is the stereochemically controlled synthesis of the carbocyclic ring system of the ingenanes that embodies an "inside-outside" or trans intrabridgehead stereochemical relationship. This unique stereochemical feature would appear to play a very important role in the biological properties of the ingenanes as Paquette has reported that the highly functionalized ingenane analog **6**, which has cis rather than trans intrabridgehead stereochemistry (the C-8 epimer of ingenol), is completely devoid of biological activity.[6] We have previously reported the application of the intramolecular dioxenone photocycloaddition to the first synthesis of the ingenane ring system, **7** (Scheme I), with the trans intrabridgehead stereochemical relationship.[7] We have now extended this work to the synthesis of more highly functionalized analogs and we report herein the synthesis and initial biochemical evaluation of saturated and unsaturated analogs of ingenol that have substantial affinity for protein kinase C. These new compounds could be the prototypes of a new class of therapeutic agents that act by controlling protein phosphorylation.

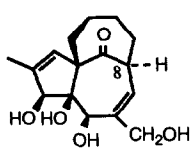
Scheme I



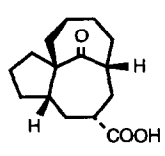
1 R = R' = H
2 R = R' = n-BuCO



3 R = R' = H
4 R = R' = PhCO
5 R = PhCO; R' = H



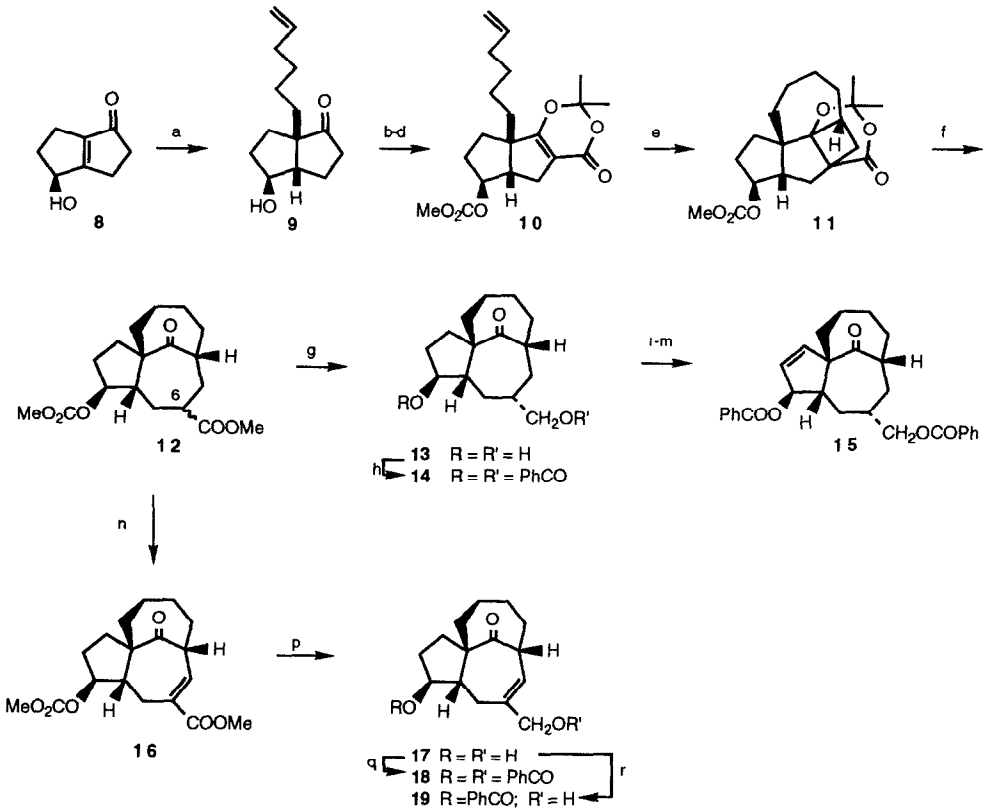
6



7

Among protein kinase C modulators, the ingenanes are both intriguing and relatively unexplored. Little difference in *in vivo* potency is observed between ingenol 3-monoesters and ingenol 3,20-diesters,[8] whereas substitution of phorbol derivatives at C-20 is strongly deactivating.[9] The parent alcohol ingenol *per se* retains protein kinase C affinity and biological activity,[10] albeit weakly, whereas the parent alcohol phorbol is entirely devoid of activity.[11] Some computer models suggest that the ester carbonyl of the ingenol ester forms part of the protein kinase C pharmacophore, as contrasted to a hydroxyl group in phorbol esters.[12] Different protein kinase C modulators have been found to range from activators to partial biological antagonists,[13] and ingenol 3,20-dibenzoate was reported to have anti-leukemic activity.[14] To further evaluate ingenane structure-activity relationships, the synthesis and biological activity of C-3, C-20-diacylated derivatives of simple ingenol analogs have been examined, the preparation of which are outlined in Scheme II.

Scheme II



a) Li/NH_3, 5-hexenyl iodide, 45-52%; b) LDA, MeOCOCN, 89%; c) p-$OMeC_6H_4CH_2OH$, 81%; d) TFAA, TFA, Me_2CO, 77%; e) hv, 61%; f) p-TsOH, MeOH, 84%; g) DIBAL-H, THF (86%); h) PhCOCl, DMAP, PhMe (93%); i) 1 equiv PhCOCl, DMAP, PhMe (87%); j) PCC (94%); k) 1. LDA, PhSeCl, 2. H_2O_2 (71%), l) DIBAL-H (88%), m) Ph_3P, DEAD, PhCOOH (82%); n) 1. KHMDS, PhSeCl (77%), 2. H_2O_2 (83%); p) DIBAL-H (74%); q) PhCOCl, DMAP, CH_2Cl_2 (90%); r) 1. TBDMSOTf, Et_3N, DMAP, CH_2Cl_2 (74%); 2. PhCOCl, DMAP, Et_3N, CH_2Cl_2 (88%); 3. $HClO_4$, MeOH/THF (83%)

Reductive alkylation of **8**[15] with 5-hexenyl iodide provided the cis-fused hydroxyketone **9** in 45-52% yield. Carboxylation of the enolate derived from **9** with methyl cyanoformate (LDA, THF, 89%) led to the formation of the desired β-ketoester, which on ester exchange (anisyl alcohol, toluene, reflux, 16 h, 81% yield) and dioxenone formation (TFA, Ac_2O, acetone, 12 h, 77%) gave **10**. Irradiation of **10** led to the formation of a single photoadduct **11** (61% yield) which on acid fragmentation (p-TsOH, MeOH, reflux, 84% yield) gave the C-3, C-20 difunctionalized ingenane **12** as a mixture of C-6 ester epimers.

Conversion of **12** into a series of C-3, C-20 diesters for biochemical testing was achieved as outlined in Scheme II. The fully saturated dibenzoate **14**, lacking both the A ($\Delta^{1,2}$) and B ($\Delta^{6,7}$) ring alkenes, was obtained by reduction (DIBAL-H, THF, 86%) and acylation (PhCOCl, DMAP, CH_2Cl_2, 93%) of **12** (C-6α epimer). The $\Delta^{6,7}$ alkene-containing **16** could be obtained from **12** by selenation (KHMDS, PhSeCl, 71%) followed by oxidative elimination (H_2O_2, H_2O, CH_2Cl_2, 83%) to give a mixture of the $\Delta^{6,7}$ unsaturated ester **16** and the $\Delta^{5,6}$ regioisomer in a ratio of 5:1. Reduction (DIBAL-H, THF, 74% yield) followed by acylation (PhCOCl, DMAP, CH_2Cl_2, 90% yield) led to the formation of **18**. The $\Delta^{1,2}$ unsaturated dibenzoate **15** was prepared from diol **13** as outlined in Scheme II.

Compounds were evaluated for their ability to interact with the regulatory site on protein kinase C, as quantitated by inhibition of [^{3}H]PDBU binding to enzyme reconstituted in the presence of phosphatidylserine and assayed for 5 min at 37°C.[16] In initial studies, the 3,20-dibenzoates **14**, **15**, and **18** were examined, together with **4**, the 3,20-dibenzoate of ingenol, **3**. Ingenol 3,20-dibenzoate yielded a K_i of 240 ± 9 nM (mean ± range, n=2) for protein kinase C-α compared to 0.54 nM for phorbol 12,13-dibutyrate, **2**. The dibenzoates **14**, **15**, and **18** were inactive.

These biochemical measurements stood in marked contrast to the *in vivo* reports that ingenol 3,20-dibenzoate, **4**, yielded an inflammatory potency in the mouse ear of 0.054 nmol/ear,[8] compared to 0.067 nmol/ear for phorbol 12,13-dibutyrate, **2**.[17] We hypothesized that the *in vivo* measurements might be misleading, since loss of the C-20 ester of the ingenol diester might occur *in vivo*, affording the more active monoester. If so, we would wish to evaluate *in vitro* the potencies of the monoesters themselves. Consistent with our hypothesis, ingenol 3-monobenzoate, **5**, yielded an apparent K_i of 0.14 ± 0.04 nM (mean ± SEM, n=3) for protein kinase C-α, a 3 order of magnitude increase relative to the corresponding dibenzoate. As an initial step, we therefore prepared **19**, the 3-monobenzoate of **17**, and found it to be indeed active with a K_i of 165 ± 21 nM (mean ± SEM, n=3). Preparation of the 3-monoesters is continuing, as is a detailed evaluation of binding affinities, kinetics, and metabolic transformation of the compounds. We can already conclude unambiguously, however, that our strategy is successful and we can prepare potent synthetic analogs in 10-15 step synthetic sequences. Further testing of these and related compounds, as well as the synthesis and biological evaluation of more highly functionalized ingenol congeners, is currently underway in our laboratory and our results will be reported in due course.

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References

[1]Recipient of the American Cyanamid Young Faculty Award (1989-1992) and a National Institutes of Health Research Career Development Award (1988-1993).

[2]Address correspondence to this author regarding the biological testing of **14**, **15**, **18** and **19**.

[3]Schmidt, R. and Aitken, A. In *Naturally Occurring Phorbol Esters*; Evans, F., Ed.; CRC Press, Inc.: Boca Raton, 1986; pp 245-289.

[4]Wender, P. and McDonald, F. *J. Am. Chem. Soc.* **1990**, *112*, 4956.

[5]For studies directed towards the synthesis of ingenanes, see a) Paquette, L.; Nitz, T.; Ross, R.; Springer, J. *J. Am. Chem. Soc.* **1984**, *106*, 1446; b) Sato, T.; Okuda, T.; Kaneko, Y.; Yamakawa, K. *Chem. Pharm. Bull.* **1984**, *32*, 1401; c) Rigby, J.; Moore, T. *J. Am. Chem. Soc.* **1986**, *108*, 4655; d) Funk, R.; Bolton, G. *J. Am. Chem. Soc.* **1986**, *108*, 4655; e) Mehta, G.; Pathak, V. *J. Chem. Soc., Chem. Comm.* **1987**, 876; f) Paquette, L.; Ross, R. *J. Org. Chem.* **1987**, *52*, 5497; g) Rigby, J.; Kierkus, P. *J. Am. Chem. Soc.* **1989**, *111*, 4125; h) Rigby, J.; Moore, T. *J. Org. Chem.* **1990**, *55*, 2959; i) Funk, R.; Olmstead, T.; Parvez, M. *J. Am. Chem. Soc.* **1988**, *110*, 3298.

[6]Paquette, L.; Ross, R.; Springer, J. *J. Am. Chem. Soc.* **1988**, *110*, 6192.

[7]Winkler, J.; Henegar, K.; Williard, P. *J. Am. Chem. Soc.* **1987**, *109*, 2850.

[8]Sorg, B. and Hecker, E. *Z. Naturforsch.* **1982**, *37b*, 748.

[9]Hecker, E. In: *Carcinogenesis, Vol. 2: Mechanisms of Tumor Promotion and Cocarcinogenesis;* Slaga, T. J.; Sivak, A.; Boutwell, R. K., Eds.; Raven Press: New York, 1978; pp 11-48.

[10]Hasler, C., Acs., G., Blumberg, P. M. *Cancer Res.* **1992**, *52*, 202.

[11]Yeh, E.; Sharkey, N. A.; Blumberg., P. M. *Phytotherapy Res.* **1987**, *1*, 135.

[12]Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. *Proc. Natl. Acad. Sci. (U.S.A.)* **1989**, *86*, 9672.

[13]Blumberg, P. M. *Molecular Carcinogenesis* **1991**, *4*, 339.

[14]Kupchan, S. M.; Uchida, I.; Branfman, A. R.; Dailey, R. G.; Fei, B. Y. *Science* **1976**, *191*, 571.

[15]The requisite hydroxyenone **8** was prepared following an unpublished procedure of Professor Eaton (University of Chicago): basic epoxidation of $\Delta^{1,5}$-bicyclo[3.3.0]octen-2-one (H_2O_2, NaOH, MeOH) followed by rearrangement of the intermediate epoxyketone with aqueous perchloric acid.

[16]Teng., K.; Marquez, V. E.; Milne, G. W. A.; Barchi, J. J. Jr.; Kazanietz, M. G.; Lewin, N. E.; Blumberg, P. M. and Abushanab, E. *J. Am. Chem. Soc.* **1992**, *114*, 1059.

[17]Thielmann, H. W. and Hecker, E. In: *Fortschritte der Krebsforschung. Vol. VII;* Schmidt, C. G. and Wetter, O., Eds.; Schattauer: Stuttgart-New York, 1969; pp 171-179.