Pure & Appl. Chem., Vol. 61, No. 3, pp. 469–472, 1989.
Printed in Great Britain.

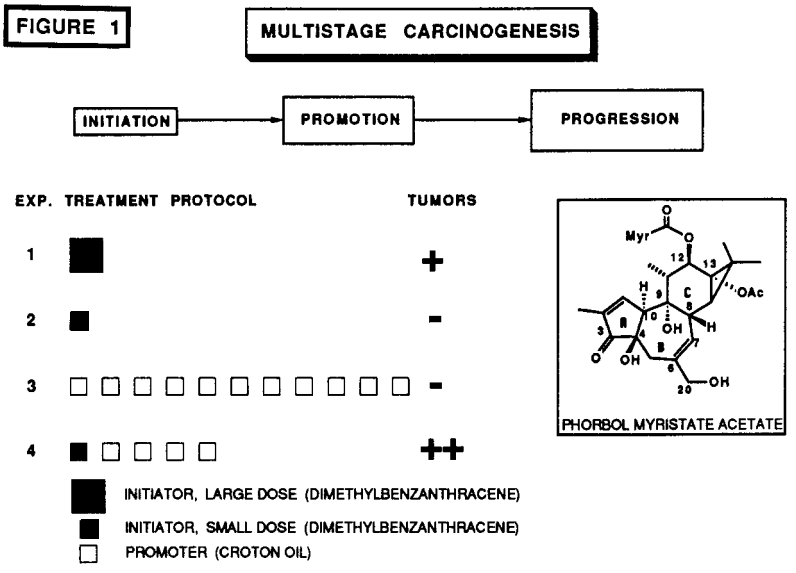
Chemistry, computers and carcinogenesis

Paul A. Wender

Department of Chemistry, Stanford University, Stanford, California 94305 USA

Abstract - This lecture describes synthetic, computer, and pharmacological studies on the molecular basis of carcinogenesis. A structural model is presented which provides a hypothesis for the similar mode of action of structurally dissimilar tumor promoters. The synthesis of rationally designed promoters is described along with the synthesis of the most potent known tumor promoter, phorbol myristate acetate.

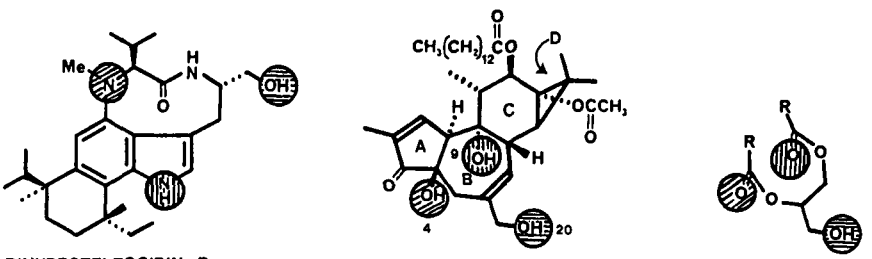
The mechanism of carcinogenesis has been a focus of multidisciplinary research for most of this century.[1] In 1914, Yamagiwa and Ichikawa[2] demonstrated that certain types of tumors can be reproducibly induced with specific agents, now commonly referred to as carcinogens. Subsequent studies revealed, however, that carcinogens are not the only risk factors involved in cancer development.[1] In the 1930's, several noncarcinogenic compounds, now referred to as tumor promoters, were shown to enhance the effect of certain carcinogens in inducing mouse skin neoplasia (Figure 1). Thus, while application of either subthreshold doses (Exp. 2) of established carcinogens (Exp. 1) or of irritants (Exp. 3) to mouse back skin resulted in no statistically significant tumor induction, the combination of these two agents (Exp. 4) resulted in an unusually high rate of carcinoma development. Later studies revealed that the most potent agent for enhancing the effect of a carcinogen is phorbol myristate acetate (PMA).[3] More recently, it has been established that the effects of PMA are in large part mediated by protein kinase C (PKC), an enzyme of great importance in cell surface signal transduction.[4] Thus, it now appears from these and extensive supporting studies that carcinogenesis is a multistage process for many types of cancer and that agents which effect the promotion stage operate at least in part through anomolous activation of PKC. In this lecture, we will describe computer modelling studies which have served to provide a structural hypothesis for how promoters like PMA are recognized by PKC and synthetic studies which have resulted in the synthesis of phorbol—the parent compound of the most potent tumor promoters yet studied.



Computer modelling studies conducted in our laboratory[5] have been directed at the elucidation of how plant derived compounds such as PMA exert their profound effect on mammalian systems. An important opportunity to understand this process occurred when Fujiki and coworkers[6] reported that certain alkaloids, while structurally very different from the diterpene PMA, potentiate similar biological responses to PMA, including mouse skin tumor promotion. Extensive modelling studies in our group have since revealed that these as well as other comparably active agents, along with the endogenous activator of PKC, namely diacyl glycerols, possess a common set of functionalities which presumably are required for recognition by and activation of PKC (Fig. 2). Further studies in this area have shown that this model extends to more recently recognized tumor promoters such as aplysiatoxin and bryostatins.[7] Significantly, we have also been able to use this model to design, for the first time, non-natural compounds which have since been shown to be competent activators of PKC.[5a] It is expected that these new agents and this predictive model will greatly expand our knowledge of the molecular role of PKC in tumor promotion as well as normal cellular signal transduction.

FIGURE 2

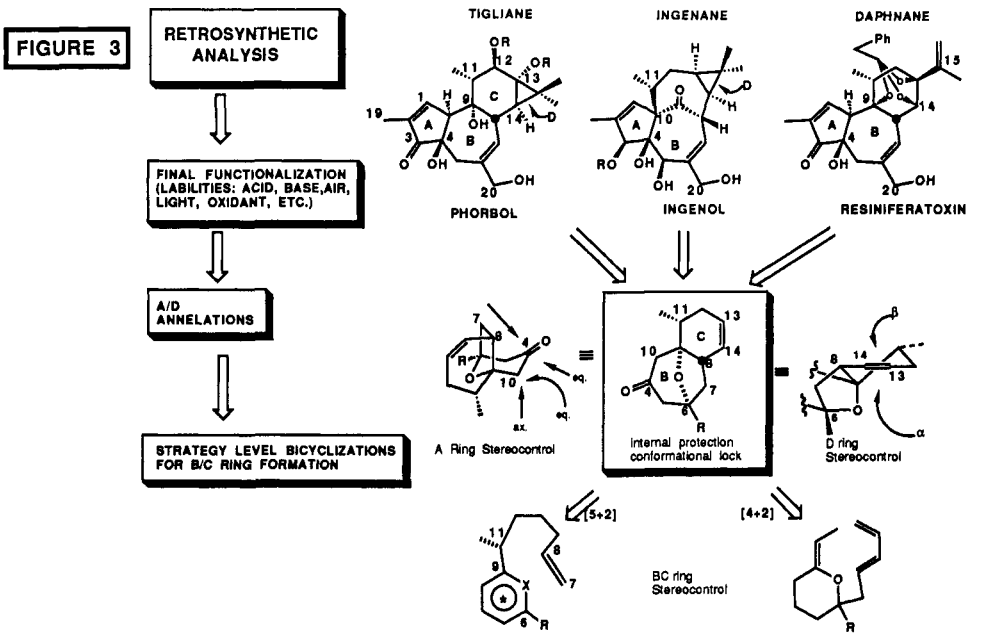
PROPOSED ACTIVATOR PHARMACOPHORE FOR PROTEIN KINASE C



DIHYDROTELEOCIDIN B PHORBOL MYRISTATE ACETATE DIACYLGLYCEROL

Concurrent with and in support of the above studies, we have embarked on a program directed at the synthesis of PMAand related diterpenes of the ingenane and daphnane families as well as their analogs (Fig. 3). It was our intent, then and now, to develop the synthetic expertise needed to address the above-noted biochemical issues while simultaneously establishing a methodology for addressing the specific and general synthetic problems posed by these diterpene promoters. In our analysis, we expected that an approach to the three biosynthetically related families of diterpenes could be fashioned through the divergent elaboration of a bicyclic system possessing the B and C rings of phorbol (Fig. 3). Since such a system, composed of fused seven-and six-membered rings, would be conformationally mobile, it was recognized that attachment of the C9 oxygen in the seven-membered ring to the C6 center would induce rigidity in this subunit, thereby allowing for the stereopredictive introduction of substituents at C4, C10, C13, and C14. Further interest in this approach derived form the opportunity it presented to explore novel [4+2] and [5+2] cycloaddition approaches to six-and seven-membered rings embedded in a polycyclic net work. A recent report detailed the successful implementation of the former strategy,[8] while our efforts on the latter will be the second subject of this lecture.

FIGURE 3

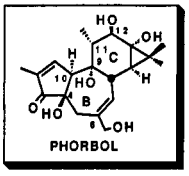


The key to our approach to the phorbol B-C ring system was based on a relatively little studied [5+2] cycloaddition of oxido-pyrylium ions with olefins.[9] Indeed, at the outset of these studies, even the factors which control stereoinduction in this homolog of the Diels-Alder reaction had not been addressed. Our plan (Fig. 4) called for the tethering of a pyrylium ion precursor to a "pyrylium-ophile" such that in the cycloaddition the tether would be expected to assume a chair-like conformation and, consequently, any tether attachments such as the C11 methyl would be preferentially equatorially oriented. Gratifyingly, the execution of this plan resulted in a completely stereocontrolled cycloaddition which allowed, in eight steps overall, for the efficient and concise synthesis of the phorboid B-C ring system.

Figure 5 describes the methodology which was deployed for the attachment of the phorbol A ring onto the cycloaddition derived B-C ring system . A noteworthy feature of this sequence is the high stereocontrol that was achieved in the generation of the C10 and C4 stereogenic centers, an expected attribute of the oxygen bridged B-C system. Thus, the C10 stereocenter arises through kinetically controlled axial protonation of a regiospecifically obtained C10-C4 enolate, while the C4 stereochemistry is attributable to the facial bias imparted by the ethano bridge on the C4 ketone π-system. Following the establishment of these stereocenters, the A-ring was then formed through a nitrile-oxide/olefin cycloaddition.

FIGURE 4

THE SYNTHESIS OF PHORBOL



FORMATION OF THE B-C RING SYSTEM

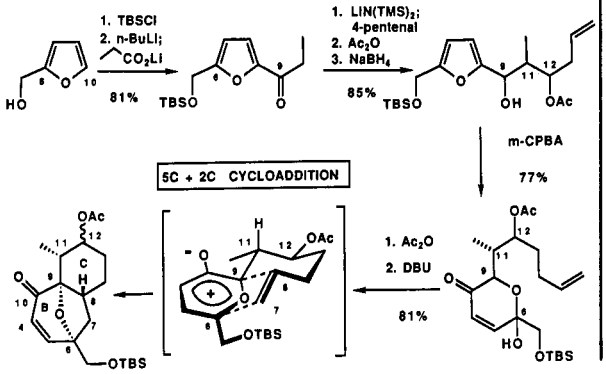
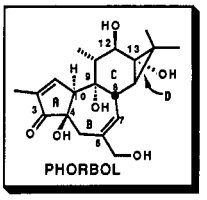
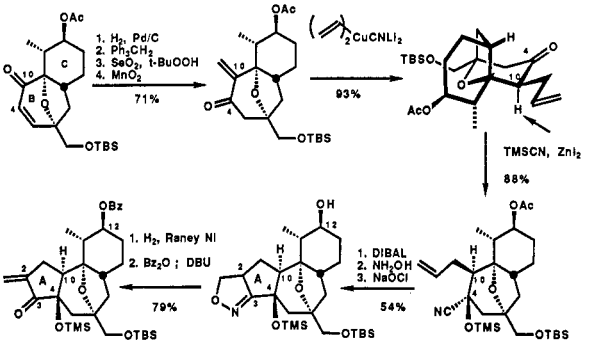


FIGURE 5

INTRODUCTION OF THE A RING

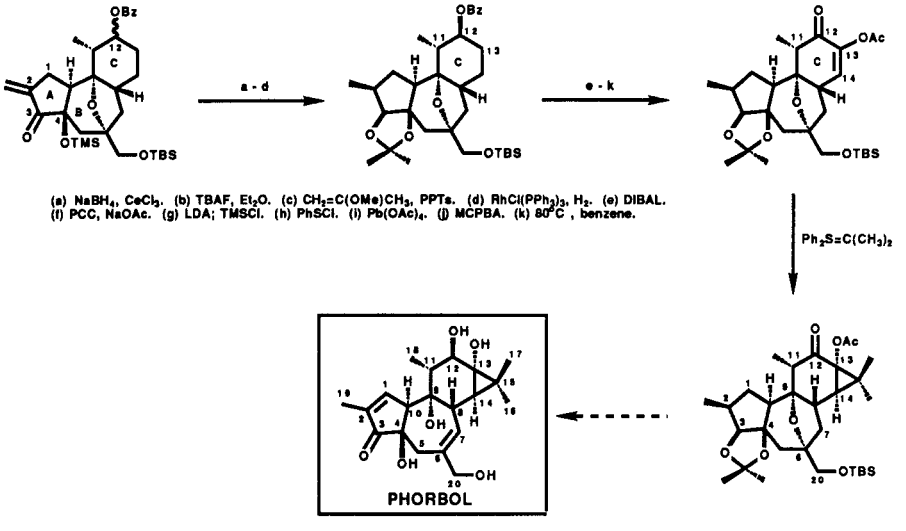




The next stage of our synthesis plan called for the introduction of the D-ring (Fig. 6). As this represented our first study on what has long been regarded as a highly reactive system (phorbol is sensitive to acid, base, oxidants, light and air), we opted for conservative protection of the A-ring functionality, after which it was expected that C-ring modifications could be more easily made. This design proved to be fruitful in that the C12 acetoxy group of our ABC tricyclic system was converted through a delicate but successful 7 step sequence into an α-acetoxy enone. As anticipated from the rigidifying effects of the oxygen-bridged B ring which creates a convex surface to the C ring beta face, cyclopropanation of the acetoxyenone proceeded in high yield to provide only the desired CD ring fusion stereochemistry. This reaction marked a major event in this synthetic area in that it produced for the first time the complete tigliane tetracyclic network.

FIGURE 6

INTRODUCTION OF THE D RING

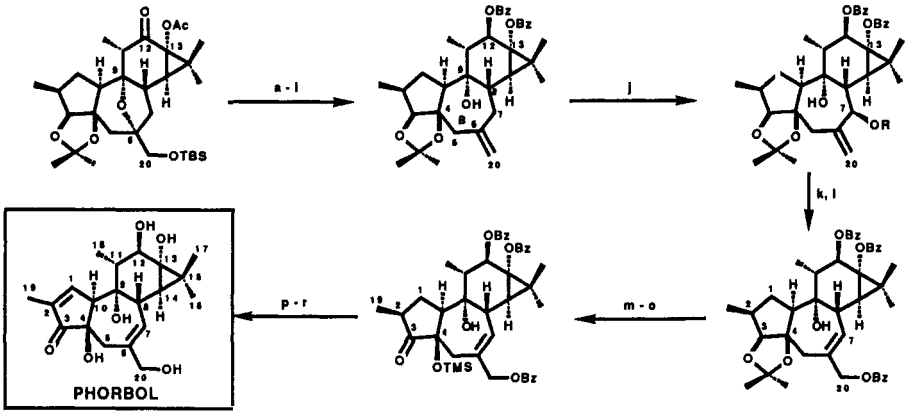


(a) $NaBH_4$, $CeCl_3$. (b) TBAF, Et_2O. (c) CH_2=C(OMe)CH_3, PPTs. (d) RhCl(PPh_3)$_3$, H_2. (e) DIBAL. (f) PCC, NaOAc. (g) LDA; TMSCl. (h) PhSCl. (i) $Pb(OAc)_4$. (j) MCPBA. (k) 80°C , benzene.

The completion of the first synthesis of phorbol followed the plan in Figure 7, conservatively fashioned in response to the sensitivity of these highly functionalized tetracycles. This subsequence required the successful execution of three objectives: cleavage of the B ring ether bridge and B ring functionalization, control of the C12 stereocenter, and A-ring functionalization. A discussion of the salient aspects of this final stage of the synthesis resulting in the synthesis of phorbol will be given.

FIGURE 7

COMPLETION OF THE SYNTHESIS



(a) DIBAL. (b) $Im_2C{=}O$. (c) TBAF. (d) Tf_2O. (e) R_4NI. (f) t-BuLi. (g) PCC, NaOAc. (h) $Na(OAc)_3BH$ (i) Bz_2O, DMAP. (j) SeO_2, t-BuOOH. (k) $SOCl_2$, propylene oxide. (l) BzOAg, BzOK, TMEDA. (m) $HClO_4$, MeOH, $HOCH_2CH_2OH$, Montmorilonite K-10. (n) $SO_3 \cdot$ pyr, DMSO. (o) MSTFA. (p) KHMDS; PhSeBr. (q) H_2O_2. (r) HF, CH_3CN.

In summary, computer modelling studies conducted in our laboratory have provided a hypothesis for the functionality requirements for PKC activation by both exogenous and endogenous PKC activators. This hypothesis has been used to design the first PKC activators which are not derived from natural activators or their analogs. Finally, the first synthesis of phorbol—the parent polyol of the most potent tumor promoters—has been completed. These efforts open new opportunities for synthesis and for the investigation of carcinogenesis and related phenomena at the molecular level.

Acknowledgements The work described in this presentation was made possible through the creative efforts of a dedicated group of coworkers: C. Cribbs, R. Keenan, K. Koehler, H.Y. Lee, J. Munger, R. Wilhelm, and P. Williams and through collaborative efforts with Dr. P. Blumberg and his group at NCI. Funding for the program was provided by the National Cancer Institute (U.S.A.) through Grant CA31841 and by a grant from Eli Lilly and Co.

REFERENCES AND NOTES

1. a) For a recent comprehensive treatment of this subject, see: "Mechanisms of Tumor Promotion, " Slaga, T.J., ed., Vol. I-IV, CRC Press, Boca Raton, Florida, **1984**. For current monographs or reviews, see: b) Hecker, E.; Adolf, W.; Hergenhahn, M.; Schmidt, R.; Sorg, B. in "Cellular Interactions by Environmental Promoters," Fujiki, H. et. al. (eds.), Japan Sci. Soc. Press, Tokyo/VNU Science Press, Utrecht, pp. 3-36, **1984**; c) Weinstein, I.B.; Galtoni-Celli, S.; Kirschmeier, P.; Lambert, M.; Hsiao, W.; Barker, J. and Jeffrey, A. in "Biochemical Basis of Chemical Carcinogenesis," Greim, et. al. (eds.), Raven Press, New York, 193, **1984**; d) Slaga, T.J. Environmental Health Perspectives **1983**, 50, 3; e) Diamond, L.; O'Brein, T.G.; Baird, W.M. in "Advances in Cancer Research" Vol. 32, Academic Press, pp. 1-74, **1980**.

2. Yamagiwa, K. and Ichikawa, K. *Gann.* **1914**, 8, 11.

3. Pettersen, R.C.; Ferguson, G. *Chem. Commun.* **1967**, 716; Hoppe, W.; Brandl, F.; Stull, I.; Rohrl, M.; Gassmann, J.; Hecker, E.; Bartsch, H.; Kreibich, G.; Szczepanski, Ch.v. *Angew. Chem.* **1967**, *79*, 824.

4. Nishizuka, Y. *Nature (London)* **1984**, 308, 693.

5. For computer modelling studies on exogenous and endogenous PKC activators and the synthesis of new activators, see: (a) Wender, P.A.; Koehler, K. F.; Sharkey, N. A.; Dell'Aquila, M. L.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4214. (b) Wender, P.A.; Koehler, K. F.; Wilhelm, R. S.; Williams, P.D.; Keenan, R. M.; Lee, H. Y. In *New Synthetic Methodology and Functionally Interesting Compounds*; Yoshida, Z. I., Ed.; Elsevier: Amsterdam, Oxford, New York, Tokyo, 1986; pp 163-182. For independent studies on exogenous PKC activators, see: (c) Jeffrey, A. M.; Liskamp, R. M. *J. Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 241. (d) Lotter, H.; Hecker, E. *Fresenius' Z. Anal. Chem.* **1985**, *321*, 640 and (e) Endo, Y; Itai, A.; Shudo, K.; Fujiki, H. *Japan. J. Cancer Chemother*, **1986**, *13*, 3365.

6. Fujiki, H.; Suganuma, M. and Sugimura, T. in "Human Carcinogenesis," Harris, C.C. and Autrup, H.N. (eds.), Academic Press, New York, pp. 303-324 (**1983**)

7. Fujiki, H.; Mori, M.; Terada, M.; Sugimura, T. and Moore, R.E. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3872. Horowitz, A.D.; Fujiki, H.; Weinstein, I.B.; Jeffrey, A.; Okin, E.; Moore, R.E. and Sugimura, T. *Cancer Res.* **1983**, *43*, 1529.

8. Wender, P.A.; Keenan, R.M.; Lee, H. Y. *J. Amer. Chem. Soc.* **1987**, *109*, 4390.

9. For related studies, see: Hendrickson, J.B. and Farina, J.S. *J. Org. Chem.* **1980**, *45*, 3359 and Sammes, P.G. and Street, L.J. *J. C. S. Chem. Commun.* **1982**, 1056.