Phorbol esters

Safety Data Sheet

Division of Occupational Health and Safety
National Institutes of Health

WARNING!

Compounds in this class are toxic and carcinogenic or co-carbonic. They are rapidly absorbed through the skin and probably the intestinal tract. They may cause severe irritation of tissues (skin, eyes, mucous membranes, and lungs) and induce sensitivity. Avoid formation and breathing of aerosols.

Laboratory operations should be conducted in a fume hood, glove box, or ventilated cabinet.

Avoid skin contact: If exposed, wash with soap and cold water. Avoid washing with solvents. Avoid rubbing of skin or increasing its temperature.

For eye exposure, irrigate immediately with large amounts of water. For ingestion, induce vomiting. Drink milk. Refer promptly for gastric lavage. For inhalation, remove victim promptly to clean air. Administer rescue breathing if necessary. Refer to physician.

In case of laboratory spill, wear protective clothing during cleanup. Avoid skin contact or breathing of aerosols. Use organic solvents to dissolve compound. Use absorbent paper to mop up spill. Wash down area with soap and water. Dispose of waste solutions and materials appropriately.

A. Background

The croton plant (Croton flavum or Croton tiglium, Euphorbiaceae is a leafy shrub, native to Southeast Asia and Caribbean Islands. Various portions of these plants have been in daily use by natives of Curacao – the roots as chewing gum substitutes, infusion of leaves as a tea, and leaves as insecticides, insect repellents, and dishwashing detergents. Leaves were also chewed for long periods as counter irritants. A high incidence of cancers of the esophagus was noted among these natives.

The fruit of the croton plant are capsules with highly toxic seeds which consist of 20% protein (including the cytotoxin crotin which causes hemolysis or hemagglutination in some species, deformation of erythrocytes in man) and 30-50% lipids. Extraction or expression of these lipids yields *croton oil*, which is toxic, a skin irritant, and a vesicant (its use as a cathartic in human and vetinary medicine was abandoned long ago). Fractionation of croton oil results in a series of long-chain glycerides and a series of *phorbol esters*. As a isolated under mild conditions these are mostly 12, 13, 20-triesters (for nomenclature see below) but change easily to 12, 13-diesters which are of major concern in this review.

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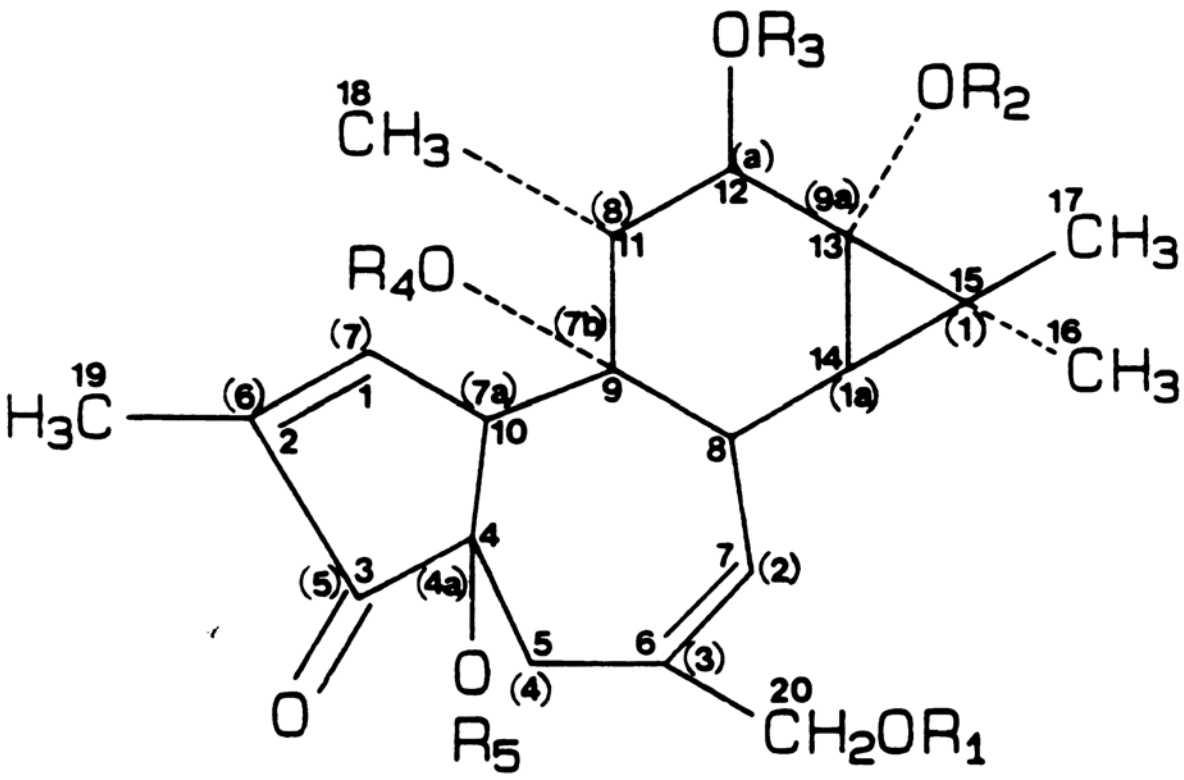
The history of the isolation of croton oil constituents and of the discovery of the co-carcinogenic properties of croton oil and of phorbol esters has been reviewed in detail (e.g., Hecker and Schmidt, 1974; Hecker, 1981).

B. Chemical and Physical Data

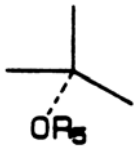
Introduction on chemical structure and nomenclature of phorbols.

Phorbol, a derivative of the parent compound tigliane, is a tetracyclic diterpene with the following structure:



The numbering system in most common use is shown by numbers without parentheses within the ring structures; thus, the compounds of greatest interest are the 12, 13-diesters and 12, 13, 20-triesters. The "official" (IUPAC, Chemical Abstracts) numbering system is shown by numbers in parentheses; it is used by relatively few authors, (*e.g.*, van Duuren and coworkers). For clarification and guidance, a few selected compounds are designated below by their official nomenclature and others may be found in their listing in the Chemical Abstract Registry.

The stereo-chemical configuration of side chains is indicated by___or ····. The only variation necessary to consider is the configuration at carbon atom 4. Phorbol itself, and most phorbol esters, have the stereo-chemical structure as shown, and is designated as "*4-β*". A few compounds under consideration have the *4-α* configuration:



(*4-α* esters have little or no biological activity). When not otherwise specified, a compound has the *4-β* configuration.

1. Croton oil: (Chemical Abstract No. 8001-28-3). A pale-yellow or brown-yellow viscous liquid with slightly disagreeable color. Density: d_{25} = 0.935-0.950. Soluble in chloroform, ether, carbon disulfide, glacial acetic acid; slightly soluble in ethanol. Decomposes on prolonged exposure to light.

2. Phorbol: $R_1 = R_2 = R_3 = R_4 = R_5 = H$. Chemical Abstract No. 17673-25-5. Synonyms: 5H-cyclopropa [3,4] benz [1,2,e]azulen-5-one, 1, 1a, 1b, 4, 4a, 7a, 7b, 8, 9, 9a-decahydro-4a, 7b, 9, 9a-tetrahydroxy-3-(hydroxymethyl) -1, 1, 6, 8-tetramethyl-, [1aR-(1aα, 1bβ, 4aβ, 7aα, 8a, 9β, 9aα)]-; $C_{20}H_{28}O_6$. Mol. wt. 363.47. Soluble in water and polar organic solvents. Anhydrous phorbol (crystallized from water) has a melting point of 250-251° C. Phorbol crystallized from ethanol and methanol retains solvent molecules tenaciously and these "alcohol phorbols" have sharp melting points in the region of 230-240° C. They are less stable than anhydrous phorbol. UV spectrum: $^{\lambda}$max ($^{\varepsilon}$max) in ethanol solution at 196 (10,600), 234 (5,060) and 332 nm (72). Data on NMR, IR, and mass spectra have been published (Hecker and Schmidt, 1974; Crombie *et al.*, 1968).

3. 12-0-tetradecanoyl-phorbol-13-acetate: $R_1 = R_4 = R_5 = H$; R_2 = n-tetradecanoyl (myristyl); R_3 = acetyl. Chemical Abstract No. 1651-29-8. Synonyms: Croton oil factor A1; phorbol 9-myristate-9a acetate; PMA; TPA.[A] $C_{36}H_{56}O_8$. Mol. wt. 616.92.

4. 4-0-methyl-12-0-tetradecanoyl-phorbol-13-acetate: $R_1 = R_4 = H$; R_2 = tetradecanoyl; R_3 = acetyl; R_5 = methyl. Chemical Abstract No. 57716-89-9. Synonym: 4-0-MTPA. $C_{37}H_{58}O_8$. Mol. wt. 630.95.

5. 12-0-retinoul-phorbol-13-acetate: $R_1 = R_4 = R_5 = H$; R_2 = retinoyl;[A] R_3 = acetyl. Chemical Abstract No. 80188-99-4. Synonym: RPA. $C_{42}H_{56}O_8$. Mol. wt. 688.

6. Phorbol-12, 13-didecanoate: $R_1 = R_4 = R_5 = H$; $R_2 = R_3$ = n-butanoyl. Chemical Abstract No. 37558-16-0. Synonym: PDBu. $C_{28}H_{40}O_8$. Mol. wt. 504.

7. Phorbol-12, 13-didecanoate: $R_1 = R_4 = R_5 = H$; $R_2 = R_3$ = n-decanooyl. Chemical Abstract No. 24928-17-4. Synonym: PDD. $C_{40}H_{64}O_8$. Mol. wt. 663.95.

8. 4*a*-phorbol-12, 13-didecanoate: R1 = R4 = R5 = H; R2 = R3 = n-decanoyl; *a*-structure at C4 (see introduction, this section). Chemical Abstract No. 27536-56-7. Synonyms: Decanoic acid, 1a, 1b, 4, 4a, 5, 7a, 7b, 8, 9, 9a-decahydro-4a, 7b-dihydroxy-3-(hydroxymethyl) -1, 1, 6, 8-tetramethyl-5-oxo-1H-cyclopropa[-3,4]benz[1,2,e]azulene-9, 9a diylester, (1a*a*, 1b*B*, 4a*a,* 7a*a*, 7b*a*, 8a, 9*B*, 9a*a*)-; 4*a*-PDD.

9. Phorbol-12, 13-dibenzoate: $R_1 = R_4 = R_5 = H$; $R_2 = R_3$ = benzoyl. Chemical Abstract No. 25405-85-0. Synonyms: PDB; PDBz. $C_{34}H_{36}O_8$. Mol. wt. 572.

10. Phorbol -12, 13, 20-tribenzoate: $R_1 = R_2 = R_3$ = benzoyl; $R_4 = R_5 = H$. Chemical Abstract No. – none. Synonym: PTBz. $C_{41}H_{40}O_9$. Mol. wt. 676. Melting point 212-213° C.

11. Phorbol-12, 13, 20-tridecanoate: $R_1 = R_2 = R_3$ = n-decanoyl; R4 = R5 = H. Chemical Abstract No. – none. Synonym: PTD. $C_{50}H_{88}O_8$. Mol. wt. 816.

12. Phorbol-12, 13-dicetate: $R_1 = R_4 = R_5 = H$; $R_2 = R_3$ = acetyl. Chemical Abstract No. 24928-15-2. Synonym: PDA. $C_{24}H_{32}O_8$. Mol. wt. 448.

[A] Underlined synonyms are abbreviations to be used subsequently in this data sheet.

13. Phorbol-20-oxo-20-desoxy-12-myristate-13-acetate: R_2 = n-tetra-decanoyl; R_3 = acetyl; $R_4 = R_5$ = H. C_{20} is CHO instead of CH_2OR_1. Chemical Abstract No. 30358-69-1. Synonyms: 20-oxo-12-0-tetra-decanoylphorbol-13-acetate; 20-oxo-TPA. $C_{36}H_{54}O_8$. Mol. wt. 614.

General chemical and physical properties of phorbol esters.

There are very few specific data in the literature. What is listed below applies, at least qualitatively, to all esters discussed in this data sheet.

1. Absorption spectroscopy: UV, IR, NMR, and mass spectra of TPA have been published (Hecker and Schmidt, 1974); they are very similar to those of phorbol and probably of all other esters. There are slight differences in the UV spectrum of 4*a* esters.
2. Volatility: No data. May be considered negligible.
3. Solubility: Phorbol esters are nearly insoluble in water: the solubility of TPA and 4*a*-PDD in phosphate-buffered saline, pH 7.4 are 2.3 mg/1 (3.7×10^{-6} M) and 1.8 mg/l (2.67×10^{-6} M), respectively (van Duuren *et al*., 1976). They are soluble in most organic solvents.
4. Description: Phorbol esters are isolated as white crystals or powders. When isolated from volatile organic solvents (ether, methylene dichloride) during fractionation of croton oil they form brittle foams which change to amorphous powders which soften below 100° C. TPA, like phorbol, strongly retains solvent molecules with which it forms addition compounds. The same probably applies to other phorbol esters as well.
5. Boiling points: No data; melting points: no data, except for phorbol and PTBz (see above). To judge by these, melting points of all phorbol esters are probably above 200° C.
6. Stability: Very sensitive to acid, alkali, elevated temperatures, light and atmospheric oxygen. Solid TPA appears to be stable when stored in the dark at -20° C, shows slow decomposition at 4° C in 3 months in the dark, and more extensive decomposition at 25° C in diffuse daylight in 3 months (the major product is the 7-hydroperoxide). It is rapidly oxidized when spread as a thin film. Solutions of TPA in DMSO may be kept at -20° C in the dark for 6 months or as a stock solution (10 mg/ml) in ethanol for 5 months in the dark under nitrogen at -5° C with essentially no decomposition. At 4° C there are only traces of decomposition, while at 25° C (in acetone, ethyl acetate, or methylene chloride) autoxidation is extensive. The main products have been identified and consist mainly of oxidation products at the double bonds (Schmidt and Hecker, 1975; Jacobson *et al*., 1975; Ohuchi and Levine, 1978).
7. Chemical reactivity: This has been reviewed (Hecker and Schmidt, 1974) for phorbol and its esters. Phorbol reduces Fehling's and Tollens reagents, and forms esters and ethers. The C_5 carbonyl group shows weak activity in reactions with carbonyl reagents but is reduced by sodium borohydribe. The double bonds are subject to reduction and to autoxidation (see Stability, above). The primary alcohol group at C_{20} is oxidized to the aldehyde with MnO_2 or CrO_3 (and also during autoxidation).
8. Flash point: No data; probably none.
9. Autoignition temperature: No data; probably none.
10. Explosive limits in air: No data; probably none.

C. Fire, Explosion, and Reactivity Hazard Data

1. Phorbol and its esters, being highly temperature sensitive, are likely to be inactivated under conditions of fire. However, because of the high biological activity of phorbol esters on the skin, and of croton oil by ingestion, it is advisable that fire-fighting personnel wear complete protective clothing and face masks.
2. Flammability is likely to be low. When handled in flammable solvents, the precautions required for such solvents will apply.
3. Conditions contributing to instability are acid, alkali, heat, and light.
4. The hazard of decomposition (autoxidation) products of phorbol esters has not been evaluated.

D. Operational Procedures

The *NIH Guidelines for the Laboratory Use of Chemical Carcinogens* describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The *NIH Guidelines* should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving croton oil or phorbol esters.

It should be emphasized that this data sheet and the *NIH Guidelines* are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: No validated method reported.
2. Decontamination: Turn off equipment that could be affected by croton oil or phorbol esters or the materials used for clean up. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 911) for assistance. Use absorbent paper to mop up spill. Wipe off surfaces with 0.1 M sodium hydroxide, and then wash with copious quantities of water. Glassware should be rinsed in a hood with ethanol or acetone, followed by soap and water. Special care should be exercised in the thorough decontamination of cages in which animals with skin exposure to active phorbol esters were housed.
3. Disposal: No waste streams containing croton oil or phorbol esters shall be disposed of in sinks or general refuse. Surplus croton oil or phorbol esters or chemical waste streams contaminated with croton oil or phorbol esters shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (*e.g.*, animal carcasses and bedding) containing croton oil or phorbol esters shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (*e.g.*, tissue cultures) containing croton oil or phorbol esters shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (*e.g.*, absorbent bench top liners) minimally contaminated with croton oil or phorbol esters shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (*e.g.*, associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing croton oil or phorbol esters shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store croton oil, solid phorbol esters, and their solutions in dark-colored, tightly closed containers or in sealed ampoules or in bottles with caps with polyethylene cone liners inside a sealed secondary container. These should be kept in a deep freeze. Avoid exposure to light and atmospheric

oxygen. Store stocks of croton oil or phorbol esters below -10° C in amber bottles with caps and Teflon cap liners in the dark and under nitrogen.

E. Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data.

2. Analysis: Very little work has been done on this subject. In distribution studies (unless the label of ^{3}H-labeled compounds is followed) the generally accepted method is ultraviolet spectrometry at 230 or 232 nm (see for instance Driedger and Blumberg, 1980 for PDBu). Since absorption spectra are essentially identical for phorbol and its esters, this method is not adequate for differentiation between them or their metabolites unless coupled with separation procedures. Separation of a variety of esters from each other and from phorbol by high pressure liquid chromatography has been described (Berry *et al*., 1977).

F. Biological Effects (Animal and Human)

Introduction

a. Reviews

This section will deal mainly with the concept and mechanism of tumor promotion by croton oil and phorbol esters. The literature on this subject is so voluminous that it can only be dealt with in summary form. Recent comprehensive review include: Weinstein *et al*., 1979; Blumberg, 1980; Slaga, 1980; Diamond *et al*., 1980; Slaga *et al*., 1982; Hicks, 1983, Blumberg *et al*., 1983. Discussions of structure-activity relationships among phorbol esters may be found in: Thielman and Hecker, 1969; Driedger and Blumberg, 1980; Diamond *et al*., 1980. Hecker (1978) tabulates activities of TPA metabolites.

b. Tumor promotion – History and mechanism

Berenblum and his coworkers, in classical experiments in the 1940s, showed that painting the skin with croton oil ("promoter") which in itself was not carcinogenic, greatly increased the yield of skin tumors in mice which had previously been treated with a low dose of benzopyrene ("initiator"). Subsequent work by Berenblum and other laboratories revealed that (1) other initiating carcinogens (*e.g.,* other PAHs) were equally effective, in a dose well below that which by itself produces tumors or indeed any morphological changes; (2) the promoter must be applied repeatedly over a period of several weeks; (3) the first promoter application may be delayed for a long time, in some experiments for as long as a year (this property distinguishes a promoter from a co-carcinogen which must be applied with , or very shortly after, and initiator); (4) croton oil may be replaced by phorbol esters with various degrees of efficiencies (see below); (5) the phenomenon of initiation and promotion is not confined to mouse skin (as was originally thought and which has been the tissue of choice in most experiments) but can be demonstrated in other mouse tissues (vagina, fore stomach, trachea, lung, etc.) and other species (hamster and guinea pig skin, rat stomach, human esophagus) and in cell cultures; (6) in general, the first tumors to appear are papillomas, followed on prolonged treatment by malignant tumors.

More recent work has established that tumor promotion consists of consecutive phases. Stage 1 is a reversible process (initial tissue hyperplasia may regress and not produce visible tumors); on prolonged application of promoters this followed by Stage 2 promotion which causes proliferation of initiated cells into visible papillomas. Finally, an irreversible process transforms preneoplastic into malignant cells. Some phorbol esters (*e.g.,* TPA) are both Stage 1 and Stage 2 promoters; others are "incomplete promoters" (*e.g.,* 4-0-MTPA is a Stage 1 promoter; RPA is a Stage 2 promoter) which function only if

initiated tissue has been treated with TPA a few times, not enough for complete promotion (Fürstenberger *et al.,* 1981; Simantov *et al.,* 1983; for a recent discussion see Slaga, 1983).

1. Absorption: Croton oil and active phorbol esters promote tumor formation when applied to initiate skin, after penetration into the epidermis. There are no data to indicate whether the same effect is produced when these materials are administered by other routes.

2. Distribution: A few studies have been carried out on the distribution of deuterated phorbol esters in animal tissues. The results of these studies should be treated with certain caution; they may be vitiated by licking of the area of application, and rubbing against cage walls because of the irritating effect of the esters. With this in mind, it may be stated that 24 hours after application of [^{3}H] TPA to mouse skin 34% was recovered, almost completely in unchanged form (Berry *et al*., 1977), which might indicate distribution to other tissues. Distribution of [^{3}H] PDBu in mouse tissues has been found to be widespread (Shoyab *et al*., 1981).

3. Metabolism and excretion: The only study in which the metabolism of TPA was investigated *in vivo*, *i.e*., after application to mouse skin, is that by Berry *et al*. (1977) referred to above. Of the amount of the ^{3}H label recovered after 24 hr., 98.5% was in the form of unchanged TPA; there was also 0.17% of 20-oxo-TPA, 0.01% of 12-tetradecanoyl phorbol and 0.01% of phorbol. All other metabolic studies were carried out using isolated skin, cell cultures, or serum. In skin, the metabolite 12-0-tetradecanoyl phospholol-13-acetate (3-OH-TPA) has been discovered (Segal *et al*., 1975). Cell cultures of Syrian hamster embryo fibroblasts deacylate TPA rapidly to phorbol-13-acetate; PDD is deacylated much more slowly but some phorbol-12-decanoate is formed (O'Brien and Saladik, 1980; Barret *et al*., 1982). Sera from rat, mouse, guinea pig, and rabbit hydrolyze TPA to phorbol-13-acetate; the sera from cow, pig, horse, chicken, and man were inactive (Lackey and Cabot, 1983). There are no data on excretory products. All evidence seems to indicate that the promoter effect (and other effects) of TPA (and other active phorbol esters?) is mediated by the intact molecule at the epidermal cell surface, with no apparent "activation" to a metabolite.

4. Toxic effects:

    a. Symptoms

    There are no data on the acute parenteral or dermal toxicity of phorbol esters, but it is judged to be moderate or low. The physiological effects of ingestion of croton oil have been summarized (Hecker and Schmidt, 1974; Dreisbach, 1983). As a very potent cathartic and irritant, it produces congestion and degenerative changes in the gastrointestinal tract, liver, kidney, and brain. Ingestion or skin application results in a burning sensation and pain in the mouth and stomach, watery or bloody diarrhea, collapse, fall of blood pressure, tachycardia, coma, and death. Eye exposure results in conjunctivitis.

    b. Action at tissue and cellular level

    The morphological effect of croton oil and of active phorbol esters is a time-dependent action. Within a few hours after topical application there is edema and erythema characteristic for inflammation and irrigation. By 24 hours leukocytes have infiltrated the epidermis, and within 1-2 days there is stimulation of mitotic activity in the basal cell layers of the epidermis, followed by keratinization. If there was only a single application of the phorbol ester, the epidermis reverts to normal appearance within two weeks; if application is repeated (*e.g*., every 3-4 days) this leads to hyperplasia. (It should be noted that the above sequence of events takes place whether or not the skin was previously "initiated."

    The biochemical effects have been the subject of several recent reviews (see Introduction to this section). Stage 1 and complete promoters (croton oil, TPA, PDBu) are bound rapidly (within seconds or minutes) and specifically to the calcium and phospholipids-binding protein

kinase C, resulting in its activation. The action of this kinase is to phosphorylate serine and threonine residues in cellular proteins involved in many cellular regulatory functions. (The structure of protein kinase C has been determined, and its mode of action reviewed by Parker *et al*., 1986.) This binding action has been demonstrated in fetal and adult brain slices (Murphy *et al*., 1983), cell cultures (Dunphy *et al*., 1980; Greenebaum *et al*., 1983), and with partially purified enzyme (Shoyab and Todaro, 1982). It is reversible, and the binding of one promoter may be competitively inhibited by another (Yamasaki, 1980). Stage 2 promoters induce enzymes whose products accelerate cell division. One of the most dramatic actions is the 200-400 fold increase in epidermal ornithine decarboxylase (O'Brien, 1976), and enzyme involved in the synthesis of putrescine, spermidine, and spermine which in turn control the synthesis of cellular RNA, DNA, and protein and therefore influence cell division. A similar effect has been noted on induction of another enzyme involved in polyamine synthesis, S-adenosylmethionine decarboxylase. Other activities, likely to be the result of these reactions (and too numerous to be discussed in detail here) include: induction of plasminogen activators in chick embryo fibroblasts in culture (Wigler *et al*., 1978), stimulation of prostaglandin synthesis in kidney cells (Ohuchi and Levine, 1978), and inhibition of epidermal growth factor binding to mouse carcinoma cells (competition for receptor sites) (Salomon, 1981).

c. Structure-activity relationships

In this review, attention is mainly focused on the relationship between chemical structure of phorbol esters and promoting ability. Many correlations have been attempted between promoter action and other physiological effects of these esters (*e.g*., irritancy); these have been reviewed *(e.g*., Thielmann and Hecker, 1969; Hecker and Schmidt, 1974).

Of all symmetrical and unsymmetrical phorbol 12, 13 diesters, those with highest activities have a combined chain length of 14 to 20 carbons; in all respects, TPA has the highest activity of all. PDD and the dioctanoate are almost as high in activity, and on either side activity drops off rapidly; PDA has very low activity in a highly sensitive mouse strain only (Baird and Boutwell, 1971), PDBz has intermediate activity. Thus, a first requirement is the presence of a lipophilic portion in the molecule. A second one is for hydrophilic centers, *i.e*., free OH groups: 4-0-MTPA is a weak promoter, as are esters at the C_{20} position. (As mentioned in the introduction, 12, 13, 20 triesters are likely to be the natural constituents of croton oil but are readily hydrolyzed at the C_{20} position.) 20-oxo TPA is intermediate in activity. Phorbol, which lacks lipophilic centers, is inactive. Finally, a third requirement is the 4-*B*structure: 4*a*-PDD (and all 4*a* analogs of the compounds under discussion, such as 4*a*-TPA) is also inactive. RPA is in a special category; it is at least as powerful an irritant as TPA but has not tumor promoting activity; however, as mentioned above, RPA is a potent Stage 2 promoter if its application is preceded by one or two applications of TPA or another complete or Stage 1 promoter. There are no data concerning PTD and PTBz; from what has been said above their activity would depend on the ease of hydrolysis of the C_{20} ester group. It should be noted that there is no evidence that this hydrolysis takes place in mouse skin.

For comparative data on other phorbol derivatives, such as monoesters and oxidation products at positions 3, 6, or 7 see reviews by Hecker (1978) and Diamond *et al.*, (1980).

5. Carcinogenic activity: There has been considerable controversy whether croton oil and/or phorbol esters can initiate as well as promote tumor formation. The affirmative reports must be treated very critically for several reasons. Initiators need to be applied in very small doses only, and are active by routes other than skin application (*e.g*., intragastric: Loehrke *et al*., 1983); therefore initiation may have been produced by atmospheric or food contaminants, or when

animals are reared in creosote-treated cages (Blumberg, 1980). As regards carcinogenesis due to croton oil, there is also the possibility of presence of an initiator in this mixture (Chouroulinkov and Lazar, 1974). Boutwell *et al*. (1957) mention "special precautions" taken to prevent exposure to carcinogen and report formation of papillomas and carcinomas in mice dermally exposed to croton oil; they conclude that croton oil is a weak carcinogen. The main proponents of a carcinogenic role for TPA have been in the laboratory of Iversen (Iversen and Iversen, 1979; Astrup *et al*., 1980; Iversen and Iversen , 1982; Astrup and Iversen, 1983), in addition to Chouroylinkov and Lazar (1974). Many of the experiments are devoid of proper controls. It will probably be extremely difficult to settle this controversy, but it appears that, at most, TPA and other phorbols are only very weak carcinogens.

6. Mutagenic and teratogenic effects: TPA is not mutagenic in the Ames test (but increases the frequency of reversion to histidine dependence induced by mutagens (Simmon, 1979; Soper and Evans, 1979) and sister-chromatid exchanges in hamster cells (Ray-Chaudhuri *et al*., 1982)).

G. Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing immediately and wash skin with soap and water. Skin should not be rinsed with organic solvents. Since croton oil and phorbol esters are readily absorbed through the skin avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Treat to prevent conjunctivitis. Obtain ophthalmological evaluation.
2. Ingestion: Drink plenty of milk. Induce vomiting. Refer promptly for gastric lavage (useful only before onset of symptoms).
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician at once. Consider treatment for pulmonary irritation.

H. References

Astrup, E.G. and O.H. Iversen. 1983. The tumourigenic and carcinogenic effect of 12-0-tetradecanoylphorbol-13-acetate when applied to the skin of BALB/Ca and hairless (HR/HR) mice. Acta Path Microbiol Scand [A], 91:103-113.

Astrup, E.G., O.H. Iversen, and K. Elgjo. 1980. The tumourigenic and carcinogenic effect of TPA (12-0-tetradecanoylphorbol-13-acetate) when applied to the skin of BALB/cA mice. Virchow's Arch B Cell Pathol 33:303-304.

Baird, W.M. and R.F. Boutwell. 1971. Tumor promoting activity of phorbol and four diesters of phorbol in mouse skin. Cancer Res 31:1074-1079.

Barret, J.C., M.T. Brown, and E.E. Sisskin. 1982. Deacylation of 12-0-[^{3}H] tetradecanoylphorbol-13-acetate and [^{3}H] phorbol-12, 13-didecanoate in hamster skin and hamster cells in culture. Cancer Res 42:3098-3101.

Berry, D.L., M.R. Lieber, S.M. Fischer, and T.J. Slaga. 1977. Qualitative and quantitative seperatation of a series of phorbol-ester tumor promoters by high-pressure liquid chromatography. Cancer Lett 3:125-132.

Blumberg, P.M. 1980. In vitro studies on the mode of action of the phorbol esters, potent tumor promoters. CRC Crit Revs Toxicol 8:153-197, 199-234.

Blumberg, P.M., K.B. Delclos, J.A. Dunn, S. Jaken, K.L. Leach, and E. Yeh. 1983. Phorbol ester receptors and the in vitro effects of tumor promoters. Ann NY Acad Sci 407:303-315.

Boutwell, R.K., D. Bosch, and H.P. Rusch. 1957. On the role of croton oil in tumor formation. Cancer Res., 17:71-75.

Chouroulinkov, I., and P. Lazar. 1974. Action cancérogéne et cocancérogéne du 12-0-tetradecanoylphorbol-13-acetate (TPA) sur la peau de souris [Carcinogenic and co-carcinogenic action of TPA on mouse skin]. C rend Acad SC Paris, Sér. D, 3027-3030.

Crombie, L., M.L. Games, and D.J. Pointer. 1968. Chemistry and structure of phorbol, the diterpence parent of the co-carcinogens of croton oil. J Chem Soc, Section C: 1347-1362.

Diamond, L., T.G. O'Brien, and W.M. Baird. 1980. Tumor promoters and the mechanism of tumor promotion. Adv Cancer Res 32:1-74.Dreisbach, R.H. 1983. Handbook of Poisoning, 11th ed. Lange Medical Publications, Los Altos, CA.

Driedger, P.E. and P.M. Blumberg. 1980. Specific binding of phorbol ester tumor promoters. Proc Natl Acad Sci USA 77:567-571.

Dunphy, W.G., K.B. Delclos, and P.M. Blumberg. 1980. Characterization of specific binding of [3H] phorbol-12, 13-dibutyrate and [3H] phorbol-12-myristate 13-acetate to mouse brain. Cancer Res 40:3635-3641.

Fürstenburger, G., D.L. Berry, B. Sorg and F. Marks. 1981. Skin tumor promotion by phorbol esters is a two-stage process. Proc Natl Acad Sci USA 78:7722-7726.

Greenebaum, E., M. Nicolaides, M. Eisinger, R.H. Vogel, and I.B. Weinstein. 1983. Binding of phorbol dibutyrate and epidermal growth factor to cultured human epidermal cells. JNCI 70:435-441.

Hecker, E. 1978. Structure-activity relationships in diterpene esters irritant and co-carcinogenic to mouse skin. In Carcinogenesis – A Comprehensive Survey. Vol. 2, Pages 11-48 Slaga, T.J., A. Sivak, and R.K. Boutwell (eds).

Hecker, E. 1981. Co-carcinogenesis and tumor promoters of the diterpene ester type as possible carcinogenic risk factors. J Cancer Res Oncol 99:103-124.

Hecker, E. and R. Schmidt. 1974. Phorbol esters – the irrigants and co-carcinogens of Croton Tiglium L Fortschr Chem Organ Naturstoffe 31:377-467.

Hicks, R.M. Pathological and biochemical aspects of tumour promotion. Carcinogenesis 4:1209-1214.

Iversen, O.H. and U.M. Iversen. 1982. Must initiators come first? Tumorigenic and carcinogenic effects on skin of 3-methylcholanthrene and TPA in various sequences. Br J Cancer 45:912-920.

Iversen, U.M. and O.H. Iversen. 1979. The carcinogenic effect of TPA (12-0-tetradecanoylphorbol-13-acetate) when applied to the skin of hairless mice. Virchow's Arch B Cell Pathol 30:33-42.

Jacobson, K., C.E. Wenner, G. Kemp, and D. Papahadjopoulous. 1975. Surface properties of phorbol esters and their interaction with lipid monolayers and bilayers. Cancer Res 35:2991-2995.

Lackey, R.J. and M.C. Cabot. 1983. Serum lipase active in the hydrolysis of the tumor promoter, 12-0-tetradecanoylphovol-13-acetate. Cancer Lett 19:165-172.

Loehrke, H., J. Schweizer, E. Dederer, B. Hesse, G. Rosenkranz, and K. Goertler. 1983. On the persistence of tumor initiation in two-stage carcinogenesis on mouse skin. Carcinogenesis 4:771-775.

Murphy, K.M.M., R.J. Gould, M.L. Oster-Granite, J.D. Gearhart, and S.H. Snyder. 1983. Phorbol ester receptors: autoradiographic identification in the developing rat. Science 222:1036-1038.

O'Brien, T.G. 1976. The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. Cancer Res 36:2644-2653.

O'Brien, T.G. and D. Saladik. 1980. Differences in the metabolism of 12-0[^{3}H] tetradcanoylphorbol-13-acetate and [^{3}H] phorbol-12, 13-didecanotae by cells in culture. Cancer Res 40:4433-4437.

Ohuchi, K. and L. Levine. 1978. Stimulation of prostaglandin synthesis by tumor-promoting phorbol-12, 13 diesters in canine kidney (MDCK) cells. J Biol Chem 253:4783-4790.

Parker, P.J., L. Coussens, N. Totty, L. Rhee, S. Young, E. Chen, S. Stabel, M.D. Waterfield, and A. Ulrich. 1986. The complete primary structure of protein kinase C – the major phorbol ester receptor. Science 233:853-866.

Ray-Chaudhuri, R., M. Currens, and P.T. Iype. 1982. Enhancement of sister-chromatid exchanges by tumor promoters. Br J Cancer 45:769-777.

Salomon, D. 1981. Inhibition of epidermal growth factor binding to mouse embryonal carcinoma cells by phorbol esters mediated by specific phorbol ester receptors. J Biol Chem 256:7958-7966.

Schmidt, R. and E. Hecker. 1975. Autoxidation of phorbol esters under normal storage conditions. Cancer Res 35:1375-1377.

Segal, A., B.L. van Duuren, and U. Maté. 1975. The identification of phorbol myristate acetate as anew metabolite of phorbol myristate acetate in mouse skin. Cancer Res 35:2154-2159.

Shoyab, M. and G.J. Todaro. 1982. Partial purification and characterization of a binding protein for biologically active phorbol and ingenol esters from murine sera. J Biol Chem 257:439-445.

Shoyab, M., T.C. Warren, and G.J. Todaro. 1981. Tissue and species distribution and developmental variation of specific receptors for biologically active phorbol and ingenol esters. Carcinogens 2:1273-1276.

Simantov, R., F. Marks, G. Fürstenburger, and L. Sachs. 1983. Control of endogenous cell regulators by the second-stage tumor promoter phorbol-12-retinoate-13-acetate. Int J Cancer 31:497-500.

Simmon, V.F. 1979. In vitro mutagenicity of chemical carcinogens and related compounds with *Salmonella typhimuria*. J Natl Cancer Inst 62:893-899.

Slaga, T.J. 1980. Cancer: Etiology, mechanism and prevention – A summary. In Carcinogenesis – A comprehensive survey. Vol. 5, Pages 243-262 Slaga, T.J. (ed).

Slaga, T.J. 1983. Overview of tumor promotion in animals. Eviron Health Perspect 50:3-14.

Slaga, T.J., S.M. Fisher, C.E. Weeks, K. Nelson, M. Mamrack, and A.J.P. Klein-Szanto. 1982. Specificity of mechanism(s) of promoter inhibitors in multistage promotion. In Carcinogenesis: A comprehensive survey. Vol. 7, Pages 19-34 Hecker, E. *et al*. (eds).

Soper, C.J. and F.J. Evans. 1977. Investigations into the mode of action of the co-carcinogen 12-0-tetradecanoylphorbol-13-acetate using autotrophic bacteria. Cancer Res 37:2487-2491.

Thielmann, H.W. and E. Hecker. 1969. Beziehungen zwischen der Struktur von Phorbolderivaten und ihren entzündlichen und tumorpromoviereden Eigenscaften [Relations between structure of phorbol derivatives and their irritant and tumor-promoting properties]. In Fortschritte der Krevsforschung. Vol. 7, Pages 171-179 Schmidt, C.G. and O. Wetter (eds). Schattauer, Stuttgart, NY.

Van Duuren, B.L., S. Banerjee and G. Witz. 1976. Fluorescence studies on the interaction of tumor promoter phorbol myristate acetate and related compounds with rat liver plasma membranes. Chem – Biol Interactions 15:233-246.

Weinstein, I.B., L.S. Lee, P.B. Fisher, A. Mufson, and H. Yamasaki. 1979. The mechanism of action of tumor promoters and a molecular model of two-stage carcinogenesis. In Environmental carcinogenesis: Occurrences, risk evaluation and mechanisms. Proc Int Conf on Environ Carcinogenesis (Amsterdam). Pages 265-285 Emmelot, P. and E. Kriek (ed). Elsevier, NY.

Wigler, M., D. DeFeo, and I.B. Weinstein. 1978. Induction of plasminogen activator in cultured cells by macroycyclic plant diterpene esters and other agents related to tumor promotion. Cancer Res 38:1434-1437.

Yamasaki, H. 1980. Reversible inhibition of cell differentiation by phorbol esters as a possible mechanism of the promotion step in chemical carcinogenesis. IARC Sci Publ 27:91-111.