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Phorbol Esters: Structure, Biological Activity, and Toxicity in Animals

Gunjan Goel, Harinder P. S. Makkar, George Francis, and Klaus Becker
Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany

Phorbol esters are the tetracyclic diterpenoids generally known for their tumor promoting activity. The phorbol esters mimic the action of diacyl glycerol (DAG), activator of protein kinase C, which regulates different signal transduction pathways and other cellular metabolic activities. They occur naturally in many plants of the family Euphorbiacaeae and Thymelaeaceae. The biological activities of the phorbol esters are highly structure specific. The phorbol esters, even at very low concentrations, show toxicological manifestations in animals fed diets containing them. This toxicity limits the use of many nutritive plants and agricultural by-products containing phorbol esters to be used as animal feed. Therefore, various chemical and physical treatments have been evaluated to extract or inactivate phorbol esters so that seed meals rich in proteins could be used as feed resources. However, not much progress has been reported so far. The detoxifying ability has also been reported in some molluscs and in liver homogenate of mice. Besides, possessing antinutritional and toxic effects, few derivatives of the phorbol esters are also known for their antimicrobial and antitumor activities. The molluscicidal and insecticidal properties of phorbol esters indicate its potential to be used as an effective biopesticide and insecticide.

Keywords Detoxification, *Jatropha curcas*, Phorbol Esters, Protein Kinase C, Tumor

The term 'phorbol' is used to describe the family of naturally occurring compounds that can be referred to as tigliane diterpenes (Evans 1986). Phorbol esters are defined as "polycyclic compounds in which two hydroxyl groups on neighboring carbon atoms are esterified to fatty acids." Several plants, such as *Sapium indicum*, *S. japonicum*, *Euphorbia frankiana*, *E. cocrulescence*, *E. ticulli*, *Croton spareiflorus*, *C. tigilium*, *C. ciliatoglandulifer*, *Jatropha curcas*, *Excoecaria agallocha,* and *Homalanthus nutans*, are reported to contain the toxic phorbols (Beutler et al. 1989). Among these plants, *J. curcas* has also been reported to possess other potential toxic compounds such as curcin and hydrocyanic acid (CRC 1977). There are several other plants that contain different derivatives of phorbol and diterpenes, such as crotonogyne, crytogonone, dimorphocalyx, duvigneaudia, fahrenheitia, maprounea, and plagiostyles (Beutler et al. 1989). Indirect exposure to such plants takes place through consumption of animal products polluted with toxic phorbol esters, such as honey collected by bees (Sosath, Ott, and Hecker 1988), meat (fish or game) captured in primitive hunting that is rendered toxic by plant materials, and meat and milk produced from the animals that feed on diets contaminated with these toxic components (Zayed et al. 1998). The active phorbol ester, TPA (4ß-12-*O*-tetradecanoylphorbol-13-acetate), was first found in the croton plant, a shrub found in Southeast Asia. The interaction of phorbol ester with protein kinase C (PKC) affects activities of several enzymes, biosynthesis of protein, DNA, polyamines, cell differentiation processes, and gene expression. The toxicity of phorbol esters has been reported on feeding plants containing these esters to various animal models, such as goat, mice, rat, and fish. The molluscicidal activity has been reported against various mollusks, indicating its potential as biopesticide.

In this review the structure, mode of action, biological activity and its relation to structure, toxic effects in animals, and approaches to detoxify feeds containing these phorbol esters, with special emphasis on phorbol esters in Jatropha, are discussed.

STRUCTURE OF PHORBOL ESTERS

The structure of the phorbol esters is dependent on the tetracyclic diterpene carbon skeleton known as tigliane. Tigliane is the fundamental alcohol moiety in the phorbol esters. Tigliane contains four rings designated as A, B, C, and D (Fig. 1). Hydroxylation of this basic structure at different positions and then ester bonding to various acid moieties results in formation of large varieties of phorbol ester compounds. The phorbol, the parent diterpene of phorbol esters, contains five hydroxyl groups with different reactivity towards acylation (Hecker and Schmidt 1974). Ring A is on the left and trans linked to the 7-member ring B. Ring C is 6-membered and cis linked to the cyclopentane D ring. The two categories of phorbols, α and ß, differ in their OH group in ring C. The placement of OH group makes the phorbol an active (ß) or inactive (α) type, which results in spatial

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Address correspondence to Dr. H. P. S. Makkar, Institute for Animal Production in the Tropics and Subtropics (480b), Department of Aquaculture, University of Hohenheim, 70593 Stuttgart, Germany. E-mail: makkar@uni-hohenheim.de

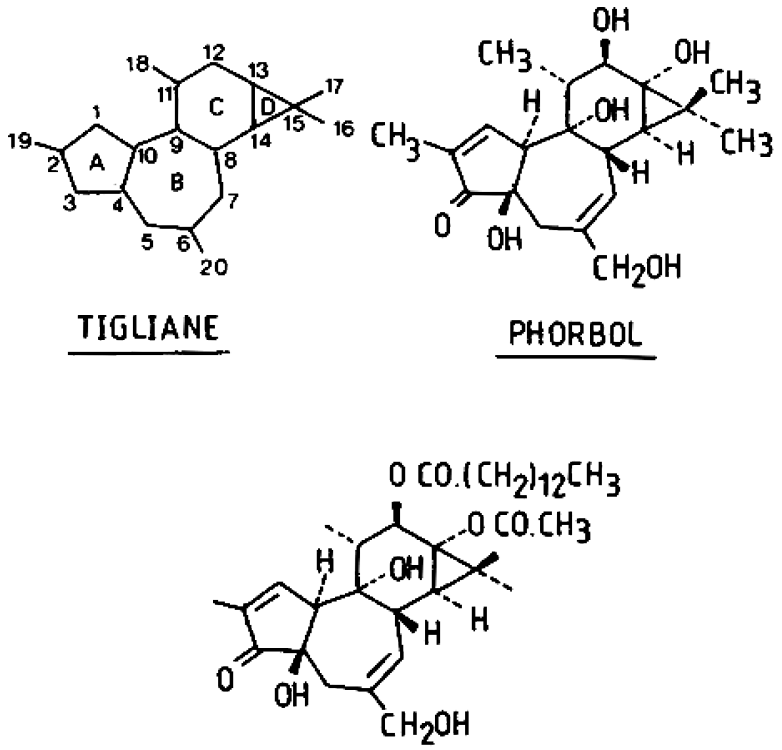


FIGURE 1
Structure of tetradecanoyl phorbol-13-acetate (TPA) (Evans 1986).

arrangement of ring D and precludes activation of PKC and other structurally similar phorbol ester receptors. The inactive α phorbol esters have similar lipophilicity and physicochemical properties as the active ß phorbols, but are unable to activate PKC due to conformational shifts (Silinsky and Searl 2003). The widely active phorbols, TPA (4ß-12-*O*-tetradecanoylphorbol-13-acetate) and PDBu (4ß-phorbol-12,13-dibutyrate) differ only by their substitutions at positions 12 and 13 of ring C. The nitrogen-containing phorbol sapintoxin A was isolated from unripened fruit of *Sapium indicum* (Taylor et al.1981). The structure of this phorbol was confirmed as 12-*O* (*N*-methylaminobenzoyl) 13-*O*-acetyl 14-deoxy phorbol. The compound was found to be a rapidly acting proinflammatory agent on mammalian skin, but was less potent than TPA. Recently, Rios and Aguilar-Guadarrama (2006) have identified four nitrogen-containing phorbol derivatives from *Croton ciliatoglandulifer*, which induces anti-inflammatory activity of ear edema in mice. The nitrogen-containing phorbol esters are restricted to members of Euphorbiaceae.

ISOLATION AND DETECTION OF PHORBOLS

The naturally occurring phorbol esters are unstable and are susceptible to oxidation, hydrolysis, transesterification, and epimerization during isolation procedures (Haas, Sterk, and Mittelbach 2002). Due to their oxygen sensitivity, the isolation must be conducted in oxygen-free conditions: solvents must be degassed and extraction should be conducted under continuous flow of nitrogen or argon. The isolation protocols involved derivatization of the functional groups in phorbol ester, mainly acylation or esterification of hydroxyl groups by chemical agents. The derivatized phorbols are then separated using different high performance liquid chromatography (HPLC) protocols. The crystallization is reported to induce loss of material due to oxidation and/or epimerization of the phorbol nucleus. The purity of isolated phorbols can be deduced using thin-layer chromatography (TLC), gas-liquid chromatography (GLC), mass spectrometry (MS), or HPLC with electrospray ionization tandem mass spectrometry (HPLC-MS-MS). The structure of these compounds is then determined using spectroscopic methods such as matrix assisted laser desorption ionization (MALDI) and nuclear magnetic resonance (NMR) techniques. Normal-phase HPLC using photodiode array detection system has been used by Dimitrijevic et al. (1999) for isolation of different tigliane and daphnane esters. Vogg et al. (1999) detected the phorbols based on collision induced fragmentation of a phorbol unit. The diterpene esters are transformed to their quasimolecular ions, which were analyzed to obtain the full-scan mass spectrum of

the compound using HPLC-MS-MS. In our laboratory, the phorbol esters have been isolated from the methanol extracts of the seed kernel and quantified using HPLC (Makkar and Becker 1997). The detection of these compounds is also based on the bioassays such as irritancy/tumor-promoting activity on mouse skin or induction of Epstein-Barr virus (EBV) in human lymphocytes and molluscicidal activities.

BIOLOGICAL ACTIVITY OF PHORBOLS

The phorbols and their different derivatives are reported to be potent tumor promoters. In addition to this effect these induce a remarkable diversity of other biological effects at exceptionally low concentration. These are responsible for skin irritant effects and tumor promotion because they stimulate PKC, which is involved in signal transduction and developmental processes of most of the cells and tissues, producing a variety of biological effects in a wide range of organisms. The inflammatory responses induced by phorbol esters are summarized in Fig. 2. Some of the major effects and the mechanisms responsible for the biological activities of phorbols are discussed below.

Action on Protein Kinase C

The primary action of phorbol esters is on biological membranes. The phorbol esters are amphiphillic molecules and have tendency to bind to phospholipid membrane receptors. These receptors are usually the primary targets for the phorbol esters. The initial membrane effects include modification in activities of cell receptors, enhanced intake of 2-deoxyglucose and other nutrients, altered cell adhesion, induction of arachidonic acid release and prostaglandin synthesis, inhibition of binding of epidermal growth factor to cell surface receptors, and altered lipid metabolism (Weinstein et al. 1979). The most investigated activity of the phorbol is its binding and activation of PKC, which plays a critical role in signal transduction pathway and regulate cell growth and differentiation (Clemens, Trayner, and Menaya 1992; Nishizuka 1992). It has been proposed that the phorbol esters convert PKC into a constitutive active form that is irreversibly inserted into the membrane (Mosior and Newton 1995). During normal signal transduction, the enzyme is activated by DAG (diacylglycerol), which is then rapidly hydrolyzed. DAG is responsible for activating PKC function by increasing its affinity for phospahtidylserine (PS)-containing membranes. Upon activation, PKC enzymes are translocated to the plasma membrane by RACK (receptor for activated C-kinase) proteins (membrane-bound receptor protein for activated PKC) to conduct various other signal transduction pathways. The phorbol acts as analogue for DAG and is a stronger PKC activator that is hardly metabolized by cell (Segal, van Duuren, and Mate 1975). It hyperactivates PKC and triggers cell proliferation, thus amplifying the efficacy of carcinogens. These phorbols can both activate PKC and after longer incubation down-regulate the enzyme (Silinsky and Searl 2003). The regulation of PKC by DAG and phorbols occur by the same mechanism, with some differences in the strength of interaction (Mosior and Newton 1995). Bryostatin, a macrocyclic lactone isolated from the marine invertebrate *Bugula neritine* (Pettit et al. 1982), also activates PKC. However, in contrast to phorbol esters, it is not a carcinogen or a complete tumor promoter (Hennings et al. 1987). Bryostatin elicits some of the same acute

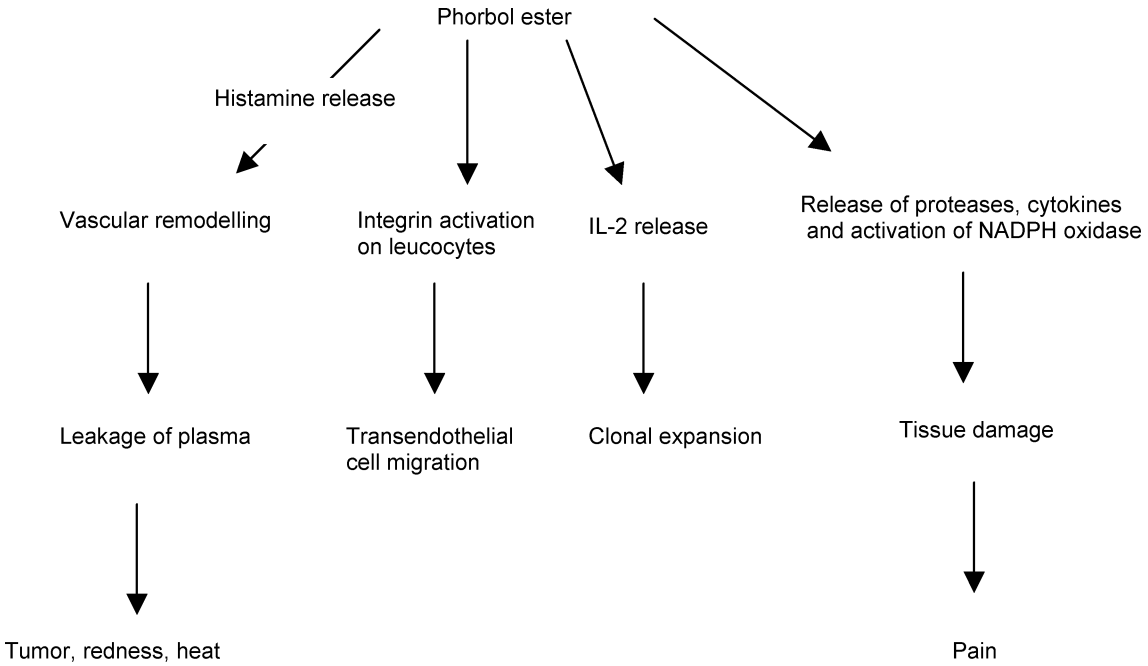


FIGURE 2
Inflammatory responses induced by phorbol esters (source: www.bioweb.wku.edu).

cellular responses as phorbol 12-myristate 13-acetate (PMA) but antagonizes chronic responses provoked by PMA (Lee et al. 1997). Bryostatin also induces the differentiation of myeloid and lymphoid cell lines, induces platelet aggregation, affects gene expression, and possesses significant antitumor activity (Amador et al. 2003).

Tumor Production

The phorbols themselves do not induce tumors but promote tumor growth following exposure to a subcarcinogenic dose of a carcinogen. They can thus be designated as cocarcinogens. The cocarcinogenic property of compounds produced by Euphorbiaceae came to light when Berenblum (1941) found that croton oil (*Croton tiglium*) was capable of enhancing tumor formation when applied to mouse skin, either together with, or separately from, a subeffective dose of the carcinogenic hydrocarbon 3,4-bezpyrene. Berebblum and Shubik (1947) found that increased tumor production was only observed when croton oil treatment followed, not preceded, the application of a carcinogen.

Although the member plants of Euphorbiacaeae have been consumed medicinally for the two millennia, these products are the most powerful tumor promoters known (Singer and Underwood 1962), as it was observed that the application of nanomolar concentration of PMA causes 100% of initiated mice to develop papillomas, with an average of 14 tumors per survivors (Hecker 1978). The phorbol esters alter the phosphorylation of specific cellular proteins and increase transcription of certain cellular genes. The mechanism behind tumor production is the interaction with PKC, which further regulates the downstream signaling pathways to induce gene transcription, leading to induction of cell proliferation. The tumor-promoting effect of phorbol esters has been studied in various human and animal cell lines.

Contradictory to this tumor-promoting ability, there are reports of apoptotic activity of the phorbol esters on tumour cells. It was observed that many malignant cell types undergo growth arrest or apoptosis in response to PKC activation by phorbol esters. The shift in activity of PKC was suggested due to its role in activating a diversity of metabolic pathways, its cellular localization, its phosphorylation, its interaction with other signal molecules, and its accessibility to different substrates (Brodie and Blumberg 2003; Gonzalez-Guerrico and Kazanitez 2005). According to Evans (1986), "the significance of plants of Euphorbiaceae and Thymelaeaceae in human cancer remains unclear at present." "Any hazard in terms of human tumor production must be less than, for example, exposure to *Nicotiana tabacum* and its products, but may nevertheless be significant as a contribution to the overall carcinogenic load of the environment." The same appears to be true even today.

Platelet Aggregation

The protein plasminogen activator is a protease, involved in the extensive reshaping of the tissues during tumor development (Reich 1978). TPA and related phorbols were reported to be a potent stimulator for plasminogen activator synthesis (Wigler, DeFeo, and Weinstein 1978). A good correlation was observed between plasminogen activator induction and tumor promotion with TPA, phorbol 12,13-didecanoate (PDD), and TPA ß-oxide (a derivative of TPA) in bioassays. However, the ability of these phorbols to aggregate human blood platelets was not equivalent to their activities in other bioassays such as skin irritancy. Therefore, the platelet aggregation of human blood platelets was suggested to be the method of choice for initial screening of the suspected tumor-promoting substances (Brynes, Schmidt, and Hecker 1980).

Cell Differentiation

The effect of TPA on cell differentiation has been extensively studied by Weinstein et al. (1979). They reported that the phorbol does not involve covalent binding to the cellular DNA, in fact it mimics the effects of transformation such as alteration in membrane morphology, increased saturation density, altered cell surface fucose glycopeptides, and increased the level of plasminogen activator and ornithine decarboxylase. They reported that TPA produces a generalized change in the physical properties of the lipid phase of the membranes, which results in increased membrane fluidity, changed cell surface morphology, and cellular adhesion. Release of arachidonic acid from membrane phospholipids was also reported which stimulated the prostglandin synthesis. The phorbols also affect terminal differentiation, which cumulatively results in production of tumors. A study conducted on the effect of TPA on HeLa cell lines reflected the blockage of G2 phase of cell cycle due to the multiple site attack of TPA on a single cell (Kinzel et al. 1984).

Metabolic Activities

The PMA has been reported to affect many enzymatic activities through its interaction with PKC. The PMA has been shown to provoke the concentration- and time-dependent decrease of mRNA coding for the enzymes such as phosphophenol pyruvate carboxykinase in H4IIE cell lines, the key enzyme in gluconeogenesis (Chu and Granner 1986). It overtakes the stimulatory effects of cAMP and glucocorticoid analogues on the transcription process. The phorbol esters also affect the activity of tyrosine hydrolxylase phosphorylation (THP), which plays a key role in catecholamine biosynthesis. The TPA phosphorylates phosphoproteins, resulting in enhancement of dihydroxypehnylalanine followed by increase in THP activity. The study was conducted in cultured bovine adrenal chromaffin cells (Pocotte and Holz 1986). Phorbol esters have also been shown to inhibit transferbinding capacity by modifying the receptors and thus limiting the uptake of iron by the cells (Testa et al. 1984). A 40% decrease in the specific binding of ^{125}I transferrin was observed for different cell lines in a dose-dependent manner (Pelicci et al. 1984).

BIOLOGICAL DETERMINANTS FOR ACTIVITY

The different biological effects of phorbols are structure dependent. The translocation of PKC to the membrane depends upon hydrophobicity of the side chain of the phorbol ester and the ability of phorbol ester to incorporate into the membrane (Bertolini et al. 2003). The phorbol ester bind to the cysteine-rich domains in PKC, which folds into globular structure, pulling apart the ß-sheets (Ron and Kazanietz 1999). The availability of biological data on a large number of derivatives and analogs of TPA permits correlation between specific atoms or functional domains and biological activities. The initial binding of a phorbol to PKC is through the oxygen at carbons 3, 4, and 20, which form hydrogen bond network with three highly conserved residues of PKC (Zhang et al. 1995). The biologically active phorbols are amphiphillic, with hydrophilic domain spanning C-3 to C-9 regions and hydrophobic region spanning C-12 and C-13 and cyclohexane- and cyclopentane-annellated ring system. The C-3, C-4 oxygens and the C-20 hydroxyl of phorbol esters play critical roles in their biological activity (Jeffrey and Liskamp 1986; Bertolini et al. 2003). The C-12 hydrophobic region is involved in relatively nonspecific hydrophobic interactions, presumably with lipids facilitating membrane insertion (Bertolini et al. 2003). The hydrophilic region is believed to play more specific roles. Overall, Jeffrey and Liskamp (1986) put forward a computerized model for different phorbols and predicted certain structural features of these esters which are important for their biological activities:

a) Polar functional groups near to O-3, O-4, O-9, O-20 of TPA;
b) The C-20 hydroxyl group must be free,
c) There should be no steric hinderance near the five membered ring; and
d) Hydrophobic moiety near C-20.

TOXICITY OF PHORBOL ESTERS

Ingestion of toxic plant material results in death of animals, reduced milk yield and reproduction, and contamination of milk with toxic constituents (Forsyth 1968). In this regard, intoxication of livestock following ingestion of many plants from the spurge genus, *Aleutrites, Jatropha, Mercurialis*, has been reported. The toxicity studies have also been reported for humans, rodents, and livestock (Adam 1974; Adam and Magzoub 1975; Ahmed and Adam 1979; Joubert et al. 1984). Toxicity of Jatropha seeds has been studied extensively in different animal models like goats, sheep, mice, rats, and fish when fed with phorbol ester–containing feeds (Adam 1974; Adam and Magzoub 1975; Makkar and Becker 1999). In most of the studies, the animals were force-fed. Decrease in the glucose level, increase in concentration of arginase, glutamate, and oxaloacetate transaminase in the serum was observed in goats with lack of appetite, reduced water intake, diarrhea, dehydration, and other hemorrhagic effects in different organs (Adam and Magzoub 1975). Adolf, Opferkuch, and Hecker (1984) isolated different phorbol derivatives from four species of Jatropha having an ID_{50} of 0.02 to 0.07 μg/ear equivalent of the standard TPA. Horiuchi et al. (1987) reported the toxicity of *Jatropha curcas* oil in mouse carcinogenesis. The irritant fraction obtained was able to induce ornithine decarboxylase and inhibit specific binding of TPA to particulate fraction of mouse skin. Along similar lines, Hirota et al. (1988) named a new phorbol from Jatropha, DHPB, a macrocyclic dicarboxylic diester, which possessed the similar activities as TPA. The compound was able to induce ornithine decarboxylase, inhibit specific binding of TPA to phorbol ester receptors, and activate PKC in vitro. But the overall biological and biochemical activities were lower than that of TPA, and its role in producing tumors in mice was found to be non significant. The effect of phorbol esters (TPA) was also studied in guinea pigs *in vivo* whereby the topical application of TPA was reported to induce inflammation and epidermal hyperproliferation by inducing DNA synthesis through prostaglandin activation and especially prostaglandin E (Bourin et al. 1982). These phorbol esters are also considered to inhibit milk secretion (Neville and Walsh 1995).

Gandhi, Cherian, and Mulky (1995) have investigated the toxicological effects of *J. curcas* (ratanjyot oil) in rats, reporting that an acute oral LD_{50} of the oil to be 6 ml/kg body weight. The oil was reported to cause toxicological manifestations like diarrhea and gastrointestinal inflammations and produced irritation followed by skin necrosis. The oil and toxic fraction also had hemolytic activity at 2.5 and 0.1 mg/ml of saline. Zayed et al. (1998) conducted a study with five different Euphorbia plant extracts from Egypt and reported them to contain toxic fractions using irritant activity and tumor-promoting activity in mice.

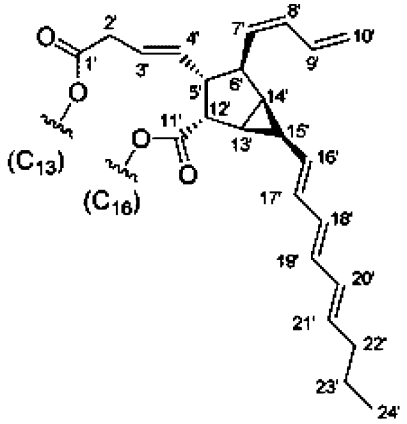
Carp (*Cyprinus carpio* L.) were found to be highly susceptible to phorbol esters present in *J. curcas*. The threshold level at which phorbol esters caused adverse effects was 15 ppm (15 μg/g) in the diet whereby a level higher than of 31 μg/g of extract in the diet resulted in lower average metabolic rate, increase fecal mucus production, and rejection of feed (Becker and Makkar 1998). Carp has been identified as a useful species for bioassay of phorbol esters, and it has been used regularly in our laboratory for evaluation of various detoxification conditions for making *J. curcas* seed meal safe for introduction in animal diets.

The toxicity of *J. curcas* to mollusks has also been investigated because of its relevance in schistosomiasis control. The phorbol esters contained in *J. curcas* were also found to be effective biopesticides against diverse fresh water snails. Snails act as intermediate hosts of schistosomes in many tropical countries. A study by Liu et al. (1997) reported molluscicidal activity of Jatropha extracts containing phorbol esters against *Biomphalaria glabrata*, *Bulinus globosus,* and *Oncomelania hupensis*. Extracts from *J. curcas* L. were also found to be toxic against snails transmitting *Schistosoma mansoni*, *S. japopnicum,* and *S. haematobium*, respectively (Liu et al. 1997; Rug and Ruppel 2000). Compared to aqueous extract, methanol extract showed the higher toxicity against all tested organisms with LC_{100} values

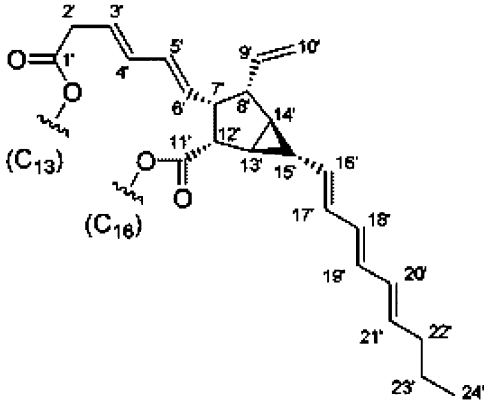
of 25 ppm for cercariae and the snail *Biomphalaria glabrata* and 1 ppm for the snails *Bulinus truncatus* and *B. natalensis*. Attenuation of cercaria larvae leading to reduced infectivity in mice could be achieved in concentrations below those exerting acute toxicity. Toxicity of Jatropherol-1 from *J. curcas* on the enzymatic activities and ultra structure of midgut cells in silkworms, *Bombyx mori,* was reported by Jing et al. (2005). The diterpene was found to be highly toxic to silkworm larvae after ingestion with LC_{50} of 0.5793, 0.2197, and 0.1578 mg/ml at 48, 72, and 120 h, respectively. The phorbol was reported to decrease the protease activities in a time- and dose-dependent manner. The dilation and shedding of ribosome from endoplasmic reticulum was observed after long exposure. Thus phorbol esters from the Jatropha plant could become an affordable and effective component of an integrated approach to schistosomiasis control.

Insecticidal activities of Jatropha oil containing phorbol esters have been reported in *Manduca sexta, Helicoverpa armigera, Aphis gossyii, Pectinophora gossypielaa, Empoasaca biguttula, Callosobruchus chinensis, Sitophilus zeamays, Phthorimaea operculella, Culex sp., Sesamia calamistis, Busseola fusca, Periplaneta Americana, Blatella germanica,* and *Oncopeltus fasciatus* (Wink et al. 1997).

Ingenol and its diesters were reported to be toxic compounds in leafy spurge (*Euphorbia escula*), which are potentially aversive to cattle, but domestic sheep and goats can consume considerable amounts of leafy spurge, although even these species may suffer a toxic response at high levels of intake (Kronberg et al. 1993; Halaweish et al. 2002). Activation of hypothalamic-pituitary-adrenal (HPA) axis was suggested to be involved in the induction of food aversions by one or more chemicals like esters in the leafy spurge which has been linked to the activation of PKC (Kronberg et al. 1993).



JATROPHA FACTOR C1

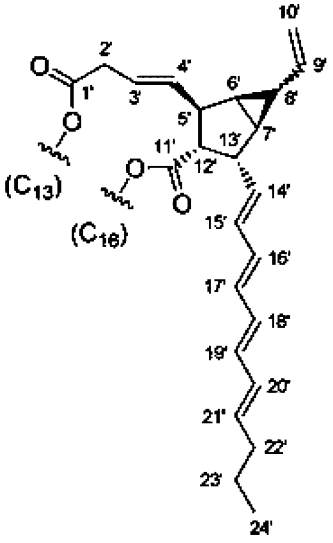


JATROPHA FACTOR C2

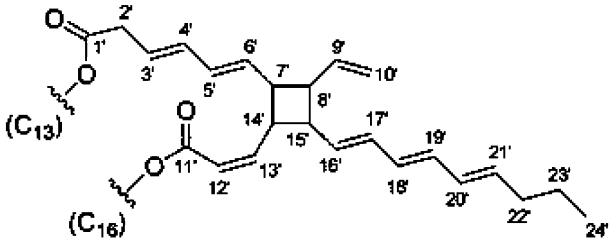
FIGURE 3
Structures of Jatropha factors (C1 to C6) from *J. curcas* (Haas et al. 2002).

PHORBOL ESTERS IN JATROPHA

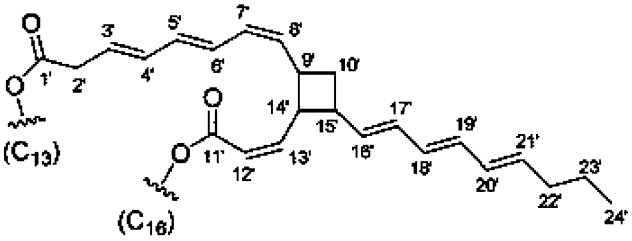
A lot of efforts are now being diverted towards utilization of agriculture waste or agricultural by-products as source of animal feed. In this regard, Jatropha has been paid a special attention as various parts/products of the plant hold potential for use as biofuel, animal feed, and inclusion in medicinal preparations. Jatropha plants have been mainly investigated as a source of oil. The seed kernel of the plant contains about 60% oil that can be converted into biodiesel and used as a substitute for diesel fuel. The seed cake remaining after oil extraction is an excellent source of plant nutrients (Foidl, Makkar, and Becker 2001). However, the presence of high levels of antinutrients prevents its use in animal feeding. Phorbol esters (phorbol-12-myristate 13-acetate) have been identified as the major toxic principle in Jatropha (Makkar and Becker 1997). The oil from the nontoxic Mexican varieties of Jatropha was reported to have negligible or low amount of phorbol esters (0.27 mg/ml of oil), whereas the toxic varieties were found to contain 2.49 mg/ml of oil. Recently, Haas, Sterk, and Mittlebach (2002) have determined the common diterpene 12-deoxy-16-hydroxyphorbol in six different diterpene esters from the *Jatropha curcas* oil using HPLC method. They named the isolated fraction as Jatropha factors C1 to C6 (Fig. 3). Factor C1 had typical bicyclohexane unit, a vinyl group, nonatrienyl residue, and single carbonyl ester chain at C-12′. Factor C2 differed from factor C1 in length of carbon chain (C-6′ in factor C1 and C-8′ in factor C2), length of ester chain connecting bicyclohexane unit with C-13 (C5′ in factor C1 and C7′ in factor C2), and the configuration at C-6′ and C-8′ in factors C1 and C2, respectively. Jatropha factors C3 and C6 were reported to have cyclobutane ring. Factor C6 differed from factor C3 in having trisubstituted cyclobutane unit rather than tetrasubstituted unit of factor C3 and in length of ester chain at C-13 of phorbol unit. The absolute stereochemistry and relative configuration of the cyclobutane unit could not be determined. Jatropha factors C4 and C5 were isolated as epimers as they were not separated by chromatography. These two units differed from factor C1 in length and position of carbon chains and orientation of bicyclohexane unit relative to phorbol. All the intramolecular diesters were reported to build from two separated monoester



JATROPHA FACTOR C4, C5



JATROPHA FACTOR C3



JATROPHA FACTOR C6

FIGURE 3
(Continued)

groups and the two dicarboxylic groups bound to the OH-13 and OH-16 of the phorbol moiety.

DETOXIFICATION OF PHORBOL ESTER–CONTAINING PLANT INGREDIENTS

There are various physical and chemical ways to destroy these phorbol esters in feeds. Solvent extraction of phorbol esters followed by heat treatment to inactivate lectins in Jatropha seed meal was reported to convert the nontoxic meal to a high-quality protein source for livestock (Makkar and Becker 1997). Makkar and Becker (1999) explored the heat-treated and untreated nontoxic variety of *J. curcas* as animal feed and reported that this variety results in a promising protein efficiency ratio (PER) and feed conversion ratio with simultaneous decrease in trypsin inhibition and lectin activities when tested in rats and fish (Carp, *Cyprinus carpio*). The PER for unheated and heated Jatropha meal–containing diet was 37% and 86%, respectively, of the casein in rats. The trypsin inhibitor and lectin activities decreased more than 83% and 99%, respectively, after 30 and 45 min of heat treatment. Heat-treated seed meal of the nontoxic variety of *J. curcas* was found to be comparable to commercially available soybean meal in nutritional quality for common carp (unpublished results from our laboratory). On the other hand, heat treatment followed by solvent extraction to remove phorbol esters could result in elimination of most of the antinutrients and toxins from the toxic variety. The meal treated in this manner was found to be innocuous to rats (Makkar and Becker 1997) and fish (unpublished results from our laboratory). The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the phorbol ester level in Jatropha seed meal to 75%. A study conducted with Jatropha seed oil reported that deacidification with sodium hydroxide and potassium hydroxide and bleaching with different agents reduced the phorbol ester level to 55%; degumming and deodorization treatments decreased phorbol esters to a lower extent (Hass and Mittelbach 2000). The degumming removed lecithin while the deodorization was used to remove the undesirable volatile and odoriferous materials. The treatment with 2.5 M KOH with 20% stoichiometric excess of base at 70°C for 20 min was able to reduce the phorbol ester content from 0.31% in degummed oil to 0.22%. It was further treated with different bleaching agents at 1% concentration for 30 min at 80°C. Trisyl type 300 was found to be the best bleaching agent, which reduced the phorbol ester content from 0.22% to 0.14%.

Hyles euphorbiae larvae are reported to detoxify TPA when administered orally; these larvae were able to metabolize nearly 70% to 90% of the phorbol ester and about 10% to 30% was retained and recovered in the feces. A potential detoxifying strategy was suggested where the larvae first store the toxic compound in an inert compartment and then by specific metabolism and detoxification, they excrete the toxin and its metabolites (Hundsdoerfer et al. 2005).

Liver carboxylesterase are reported to detoxify the phorbols (Mentlein 1986). Different cellular fractions such as the microsomal and cytosolic fractions have been reported to hydrolyse TPA. Berry et al. (1978) reported the hydrolysis of C-13 ester linkage, whereas Shoyab, Warren, and Todaro (1981) reported the hydrolysis of C-12 ester group from microsomal and cytosolic fractions, respectively. Mentlein (1986) reported two esterases, acylcarnitine hydrolase and diacylglycerol lipase from rat liver homogenate, possessing TPA hydrolyzing activity. The hydrolysis products of TPA, 12-*O*-tetradecanoyl phorbol (TP) and phorbol acetate (PA), make TPA inactive as tumor promoters (Mentlein 1986).

BENEFICIAL EFFECTS OF PHORBOL ESTERS

Phorbol esters are double-edged swords; besides having lots of negative effects on human and livestock, these also possess some beneficial effects. Some naturally occurring phorbols are tumor inhibitors, inhibit human immunodeficiency virus (HIV) replication, and possess antileukemic activity. Jatrophone, a macrocyclic diterpenoid isolated from *Jatropha gossypifolia* showed significant inhibitory activity against cancer cells under in vitro and in vivo conditions (Kupchan, Sigel, and Matz 1970). Jatrophone also possessed significant antileukemic activity against P-388 lymphocytic leukemia at 27 and 12 mg/kg cytotoxicity (ED_{50}) against KB cell culture at 0.17 μg/ml. Handa et al. (1983) reported two cytotoxic diterpenes from the stems and fruits of *Ostodes paniculata*, the chloroform extract of the plant possessed the antileukemic activity ($ED_{50} \leq 4$ μg/ml) when tested against P-388 lymphocytic leukemia cell culture system. Anti-HIV-1 phorbol esters from *Croton tiglium* induced cytopathic effects (CPEs) (El-Mekkawy et al. 2000). TPA was reported to be the only potent inhibitor of HIV-1–induced CPEs, with an IC_{100} value of 0.48 ng/ml. TPA was also reported to produce structural changes in parasite *Leishmania amazonensis* at a concentration of 20 ng/ml, whereas two other phorbols from Euphorbiaceae, namely jatrogrossidione and jatrophone, were shown to possess toxic activity against the promastigote forms of *L.brazilensis*, *L.amazonensis* and *L. chagasi,* at IC_{100} of 0.75 and 5.0 μg/ml, repectively (Chan-Bacab and Peña-Rodríguez, 2001). Chumkaew et al. (2003) evaluated the antimycobacterial activity of hexane extracts of phorbols isolated from *Sapium indicum*. They reported seven phorbol derivatives with antimycobacterial activity with a minimum inhibitory concentration (MIC) between 3.12 and 200 μg/ml, whereas two of the compounds were inactive even at MIC $>$ 200 μg/ml. The use of phorbol ester as biopesticide and insecticide has been discussed in the previous section.

CONCLUSIONS AND FUTURE RESEARCH

The phorbol esters play a key role in activation of PKC, which triggers various cellular responses, thereby resulting in inflammatory responses and tumor development. The presence of phorbol ester in some plants of agroeconomic importance, such as Jatropha, limits their use as animal feed. The force feeding of plants containing phorbol esters in animal models indicates the toxicity of phorbol esters and demands development of new methods to inactivate phorbol esters or to bring them to below threshold toxicity limits. Therefore, detoxification or complete removal of phorbol esters is essential before its use in industrial or medicinal applications or use as animal feeds. The scanty reports on the chemical detoxification of these toxic fractions necessitate improvement in physicochemical approaches to inactivate the phrobols through structural modification or complete removal. Identification and development of microbial consortia or enzymes that could degrade these compounds in feeds, making them suitable for animal feeding, could be another approach to alleviate the effects of phorbol esters. But before using them in agriculture (as biopesticide or insecticide) or health control (antimicrobial or antitumor), the fate of phorbol esters in water, soil, and plants and the potential environmental risks should be assessed.

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