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The Structural Relationship of Phorbol and Cortisol: A Possible Mechanism for the Tumor Promoting Activity of Phorbol

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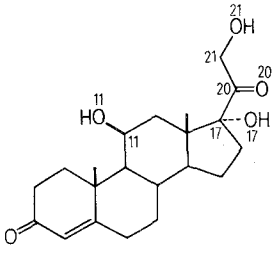
Summary. The crucial functional groups of phorbol-12-myristate-13-acetate (PMA) are superimposable with those of the glucocorticoid cortisol in a unique manner which suggests a mechanism for PMA activity, i.e. the alkylation of a cortisol 'receptor'.

Croton oil and its active principle phorbol 12-myristate, 13-acetate (PMA) causes inflammation, hyperplasia, and tumor promotion in mouse skin[1,2]. In addition PMA is the most potent platelet aggregating agent known[3], exhibiting activity at 10^{-9} *M*. The high specificity of PMA for target cells and the fact that minor structural changes in PMA eliminate its activity prompted Boutwell[4] to ask: 'Why should the plant product PMA have a specific receptor on a mammalian cell–unless PMA is mimicking the action of a natural endogenous product whose structure is similar to PMA'.

We would like to suggest that this is indeed the case and the natural endogenous product which PMA resembles is one likely to possess a crucial role in cell growth control-cortisol.



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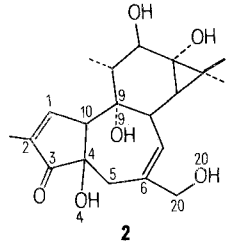

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Inhibition of croton oil promoted tumorigenesis by cortisone has been known for some time[5–7]. More recently a number of other antimitotic and anti-inflammatory steroids have been assayed for their inhibition of tumor promotion by croton oil[8] and PMA[9]. The antiinflammatory steroid Dexamethasone was shown to be the most potent inhibitor of PMA activity. Recent work has shown that PMA induces specific proteases in certain cells[10], and a correlation has been drawn between plasminogen activator production and transformed cells. Weinstein has reported that glucorticoids inhibit[11] plasminogen activator and PMA is a potent inducer[12] of plasminogen activator. Let us carefully scrutinize the structures of the cortisol **1** and phorbol **2** molecules.

No apparent structural relationship of the steroid hormone cortisol **1** to the tumor promoter phorbol **2** can readily be seen, indeed the 2 substances appear to be profoundly different. Recalling, however, that the functionality in the cortisol C and D rings are necessary for glucorticoid activity and that the functionality in the phorbol A and B rings are required for co-carcinogenic activity, we have fit the complementary cortisol and phorbol atoms using a least square program[13] with published X-ray data[14,15]. The results are displayed in the Figure. An acceptable fit (Table) was found for the crucial functionality of phorbol and cortisol. Moreover, the juxtaposition of the phorbol C(1) carbon atom with the cortisol O(20) oxygen atom suggests a mechanism of phorbol action: the alkylation of a cortisol receptor, possibly one involved in a key role in controlling cell division.

Biological nucleophiles such as an amino[16] or sulfhydryl residue[17,18], normally hydrogen bonding to the cortisol

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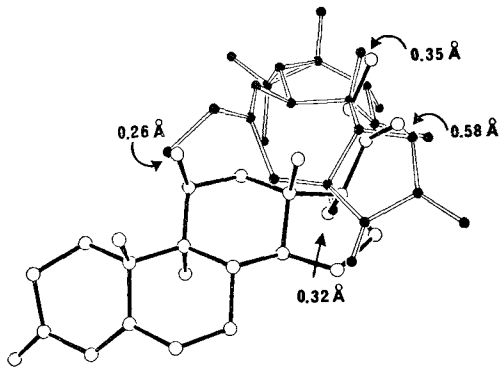
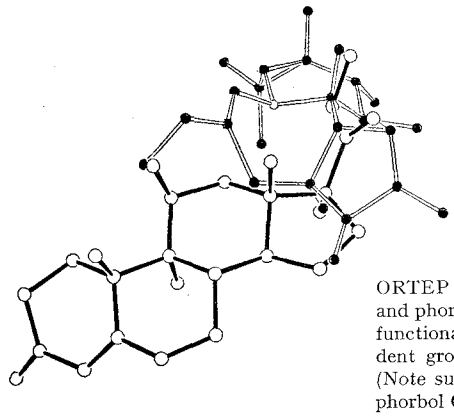
O(20) carbonyl oxygen would readily add to the reactive C(1) atom of phorbol in a 'Micheal-type' addition reaction. Such a mechanism has been suggested for the antitumor action of α-methylene lactones[19].

As additional support for the relationship of cortisol and phorbol, we have determined that phorbol (1% cross reaction) and PMA (6% cross reaction) are 'recognized' as cortisol at ca. 10^{-8} *M* in a radioimmunoassay[20,21].





ORTEP Stereopair of 9α-fluoro-cortisol ○ and phorbol ● showing overlap of crucial functional groups. Arrows indicate coincident groups with distance in angstroms. (Note superposition of cortisol 0(20) and phorbol C(1).)

The molecular similarity of 9α-fluoro-cortisol **1** and phorbol **2**

	Cortisol atom[14]	Phorbol atom[15]	Delta (Å)
Least squares fit of these atoms	O(17)	O(4)	0.322
	O(21)	O(9)	0.348
	O(20)	C(1)	0.577
	O(11)	O(20)	0.263
Unmatched atoms	C(13)	C(5)	0.533
	C(17)	C(4)	0.472
	C(20)	C(10)	0.346
	C(16)	C(3)	0.498
	C(12)	C(6)	1.546
	C(21)	C(9)	0.239
	C(11)	C(20)	2.309

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Dehydroacetic Acid in Anthers of *Solandra nitida* (Solanaceae)

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Summary. The chloroform extract of anthers of *Solandra nitida* contains 3-acethyl-6-methyl-1,2-pyran-2,4(3H)dione (dehydroacetic acid) as one of the main products.

The size of certain tropical flowers allow the isolating of chemical compounds from the different parts of the flower. We have collected and extracted a quantity of anthers of *Solandra nitida* (solanaceae) called 'golden cup'. The first compound that was found was saccharose from the methanolic extract[1] besides hexaeicosanol, 8-pentaeicosanol and nonaeicosane[2]. Now we report the presence of dehydroacetic acid.

1 kg of anthers were extracted with hexane and then with chloroform. By TLC the chloroform extract showed 14 spots in toluene-ethyl formiate-formic acid (5/5/1). By column chromatography in the same eluent, we got 13 different fractions. The 8th fraction was crystallized from toluene-AcOEt (5/5) and gave yellow needles, m.p. 100–104 °C, recrystallization in AcOEt–MeOH (1/5) gave amber needles m.p. 109–110 °C. The MS showed that the compound has 8 carbon atoms (M+1 = 8.5%)[3], molecular weight 168 and formula $C_8H_8O_4$. The IR-spectrum showed the following bands; 3065 cm^{-1} (w) CH=C, 1720 (s) C=O lactone and diketone, 1450 (m) C=CH, 1375 (m) CH_3, 1255 CH_3–C=O O=C –O–C, 1000 (s) C=CH out of plane, 860 (m)–O–C–CH_3, 780 (m) and 712 (w) C=CH st. UV-spectrum showed 2 bands at λ 223 nm (log E = 3.9) and λ_{max} at 308 nm (log E = 1.2). The NMR-spectrum showed 3 signals at δ in ppm 2.33 (s) (3H) CH_3–CO, 2.66 (s) (4H) $CH_3C(O-)=C$ and $-\overset{|}{C}-C(=O)-\overset{|}{H}$, 5,95 (m) (1 H) vinylic proton. All these data suggest that the compound is 2,6-dioxo-2-methyl-5-acethyl-dihydropyrane (dehydroacetic acid). The mass spectrum confirms this structure because we observe the following fragments; *m/e* 168, 153, 125, 110, 69, 56, 43 (base peak).

To our knowledge it is the first time that dehydroacetic acid is found in nature, but it is well known as the dimerization product of ethyl acetoacetate. The fact that it was found in the masculine sexual organ of the flower (anthers) suggests a metabolic activity related to the reproduction, e.g. a way for anabolism different to the well-known one through mevalonic acid.

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