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CONFORMATIONAL ANALYSIS OF PHORBOL ESTERS MONOLAYERS

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SUMMARY : The configuration and orientation of tumor-promoting and biologically inactive phorbol esters at a simulated lipid-water interface were established by conformational analysis. Selected conformers were then examined by a new computation method to form clusters of molecules representative of a monolayer arrangement. The results of this theoretical analysis were in good agreement with experimental data obtained in pure monolayers of phorbol esters spread at an air-water interface, and may help to elucidate the determinants of their biological potency.

Certain phorbol esters are potent tumor promoters (1, 2). Under suitable experimental conditions, the relative biological potency of distinct phorbol esters parallels their capacity to interact with phospholipids (3 - 5) and to interfere with ionophore-mediated calcium transport in model membranes (6, 7). Such interaction and interference could thus represent early events in the biological response to these agents (8). The latter view is supported by the observation that the binding of phorbol esters to liposomes formed of phospholipids closely resembles, both qualitatively and quantitatively, their specific binding to biological material (9). In order to gain further information on the behaviour of phorbol esters at the membrane level, we have investigated in the present study, by conformational analysis, the configuration and assembly of both tumor-promoting and biologically inactive agents at a simulated lipid-water interface.

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METHODS

Four distinct phorbol esters were examined, namely phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-didecanoate (PDD), 4α-phorbol 12,13-didecanoate (4α-PDD) and phorbol 12,13-dibutyrate (PDB). Phorbol is the trivial name for 4,9,12,13,20 pentahydroxy-1,6 tigliadien-3 one. In the conformational analysis of PMA, PDD or PDB the phorbol backbone was designed in the light of a previous study (10) with 4-OH cis, 8-H cis, 9-OH trans, 10-H trans, 11-CH_3 trans, 12-OH cis and 13-OH trans. In the phorbol backbone of 4α-PDD, as distinct from the other phorbol esters, 4-OH is trans. The computation approach used to predict the configuration of the phorbol ester monolayer consisted in a two-step procedure. First, the conformation of the isolated molecule of each ester and its orientation at a simulated lipid-water interface were established by a method described elsewhere (11, 12). Briefly, the total conformational energy was calculated from the Van der Waals, torsional and electrostatic energies. The latter was calculated for a dielectric constant of 16, a value intermediate to those currently used for the aqueous and hydrophobic phases at the simulated interface (12). Selected conformers were then submitted to a simplex minimization procedure (13) and their orientation at the interface eventually defined by calculations of the hydrophobic and hydrophilic gravity centers (14).

In the second step of the procedure, the assembly of molecules in the monolayer was computed as follows. The position of a molecule B relative to a reference molecule A was assessed by step-wise and successive changes of the five following parameters: the distance between the hydrophilic centers of A and B (from 0.05 to 5 nm, by steps of 0.05 nm each), the rotation of molecule B around its own Z axis (by steps of 30° each), the gravitation of molecule B around molecule A (also by steps of 30° each), the up-and-down migration of molecule B along the Z axis perpendicular to the lipid-water interface (by steps of 0.05 nm each), and the oscillation of molecule B around its Z axis (by steps of 2°30' each). In each case, the interaction between molecules A and B was calculated from the Van der Waals and electrostatic energies. The configuration of the A and B pair yielding the lowest energy was then used as reference to assess the position of a third molecule C. The same procedure was then repeated up to a total of 5 molecules, at which point the mean molecular area was found to reach a fairly stable value. When the configuration of the cluster of 5 molecules had been established, the mean molecular area was calculated from both the area occupied by each molecule and the intermolecular area, which were estimated after projection on the X-Y plane and using a grid of squares, each with a 0.1 nm side (14). Calculations were made on a CDC-CYBER 170 computer coupled to a Benson drawing table (Computing Center of Brussels University).

RESULTS AND DISCUSSION

The most probable conformers derived from the analysis of the isolated molecule of each phorbol ester were taken as those yielding the lowest internal energy, such a selection being based

on a statistical weight (Boltzmann) of all configurations (14). The most probable conformers are listed in Table 1. Other conformers yielded a probability of existence below 8 %. For PMA, two conformers with a probability of existence of 60 and 20 %, respectively, were selected for study in the monolayer model. As illustrated in Figs. 1 and 2, these conformers yielded monolayers in which the myristoyl chains were located in the hydrophobic domain, the phorbol ring being tilted relative to the interface plane. The molecular areas were not vastly different in these two models.

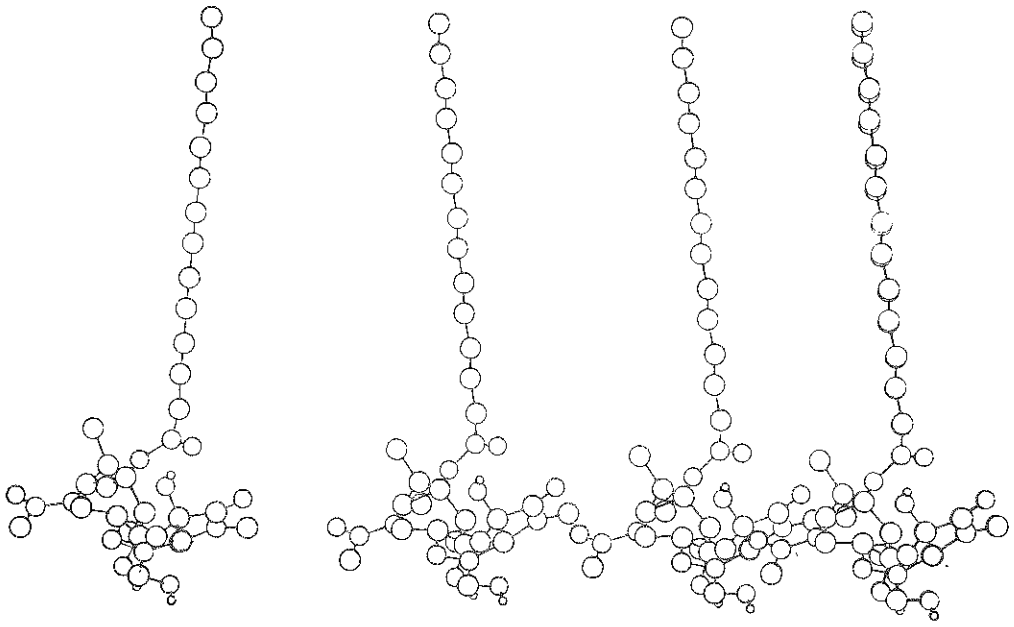

Fig. 1. Configuration of a PMA monolayer. Five molecules of the most probable conformer of PMA are shown in a frontal view, the hydrophobic domain corresponding to the uppert part of the figure. Two molecules are located one behind the other at the right end of the figure.

Table.1. Most probable conformers of four phorbol esters

Agent		PMA		PDD	4α-PDD		PDB	
Probability (%)		60	20	99	74	8	56	10
Torsional angles of acyl chain (degree)								
on C_{12}	C_{12}-O	286	284	250	290	270	280	204
	O -C_1	202	202	0	168	153	204	124
	C_1 -C_2	113	111	130	252	243	140	180
on C_{13}	C_{13}-O	172	178	106	176	178	179	173
	O -C_1	168	301	310	130	282	291	313
	C_1 -C_2	-	-	310	305	121	82	274
Energy (kcal/mole)								
hydrophobic transfer		82.1		91.9	91.9		62.7	
hydrophilic transfer		25.8		25.8	25.8		25.8	
interaction		7.8	7.2	12.9	11.0	14.0	3.2	4.2
Distance between hydrophilic and hydrophobic centers (nm)		0.325	0.458	0.525	0.257	0.480	0.130	0.126
Molecular area (nm^2/molecule)		0.64	0.59	0.69	1.15	0.60	0.72	0.52

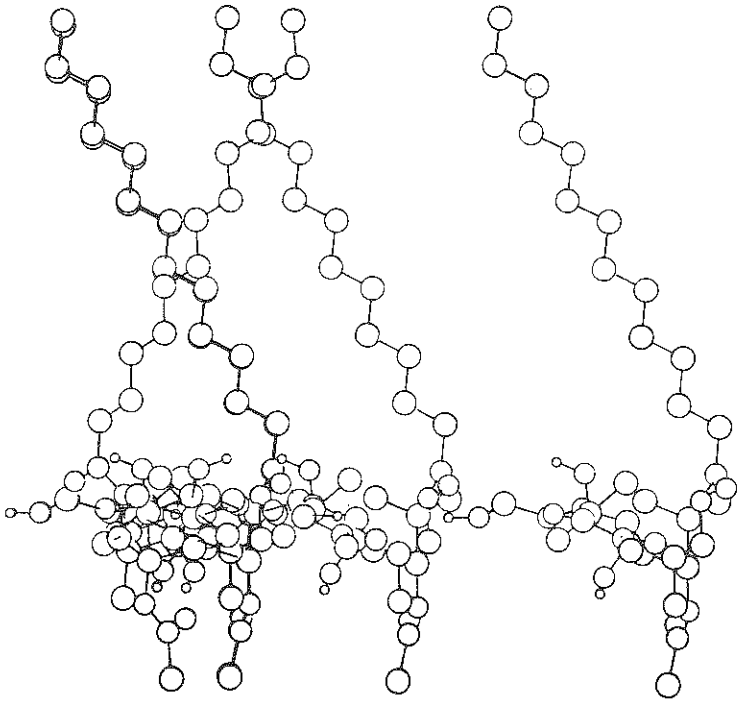

Fig. 2. Configuration of a PMA monolayer established for the conformer with a 20 % probability. Same presentation as in Fig. 1.

In the PDD monolayer (Fig. 3), the two decanoyl chains of each molecule were close to one another and more or less parallel. A different situation was found with the biologically inactive agent 4α-PDD. With the most probable conformer of 4α-PDD, the two decanoyl chains were distant of one another and oriented in divergent directions (Fig. 4). In this model, the molecules in the monolayer were arranged in a regular, almost crystal-like pattern, the molecular area being greater and the interaction energy between molecules lower than in the PDD monolayer.

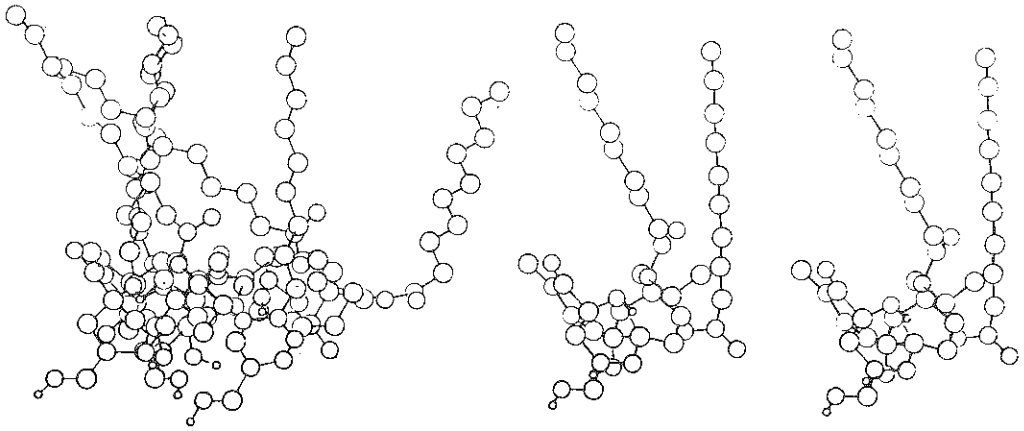

Fig. 3. Configuration of a PDD monolayer. Same presentation as in Fig. 1.

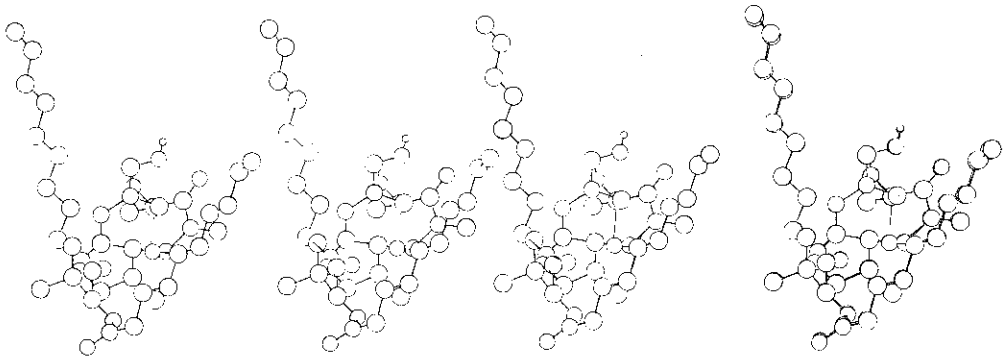

Fig. 4. Configuration of a 4α-PDD monolayer established for the conformer with a 74 % probability. Same presentation as in Fig. 1. Two molecules are located one behind the other at the right end of the figure.

However, when the monolayer was established for a less probable conformer of 4α-PDD, all decanoyl chains were now oriented towards the hydrophobic domain, whereas the phorbol four-ring system swung from a position almost parallel to the interface (Fig. 4) into an almost perpendicular position (Fig. 5).

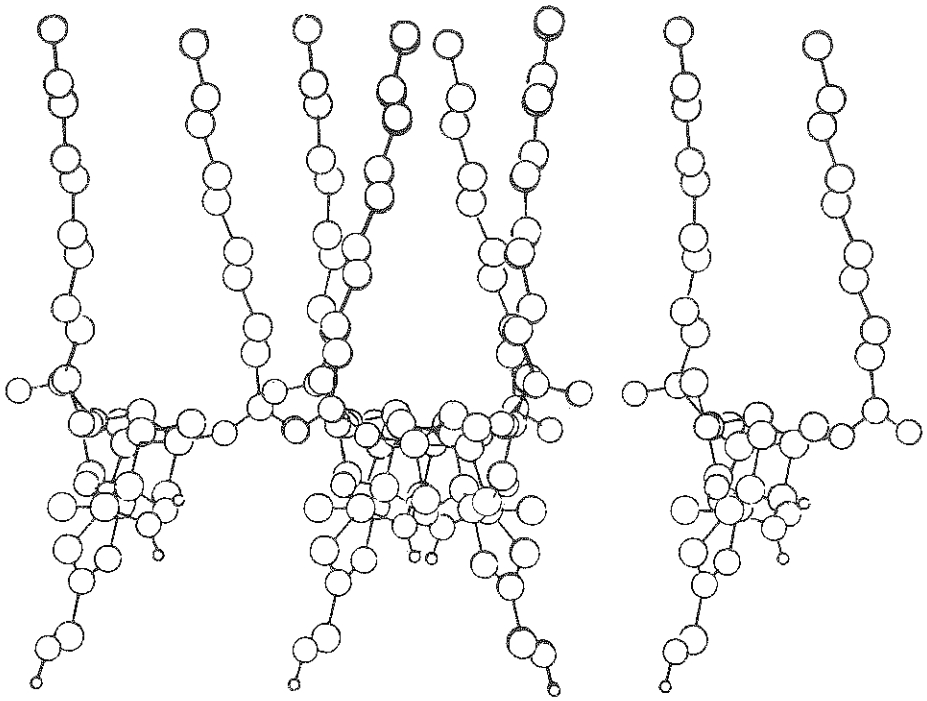

Fig. 5. Configuration of a 4α-PDD monolayer established for a conformer with a 9 % probability. Same presentation as in Fig. 1.

Last, in the PDB monolayers, the molecules displayed a more globular appearance (Fig. 6) and a low interaction energy (Table 1).

As already mentioned, the molecular area of PDD, as well as those of PMA conformers, were strikingly lower than that of the most probable conformer of 4α-PDD (Table 1). These data coincide with experimental measurements performed at an air-water interface and indicating that, at low surface pressures (< 10 dyne/cm), the molecular area of 4α-PDD exceeds that of PMA or PDD (3, 5, 15). At higher surface pressures (e.g. 15 to 20 dyne/cm), however, these three phorbol esters display closely similar molecular

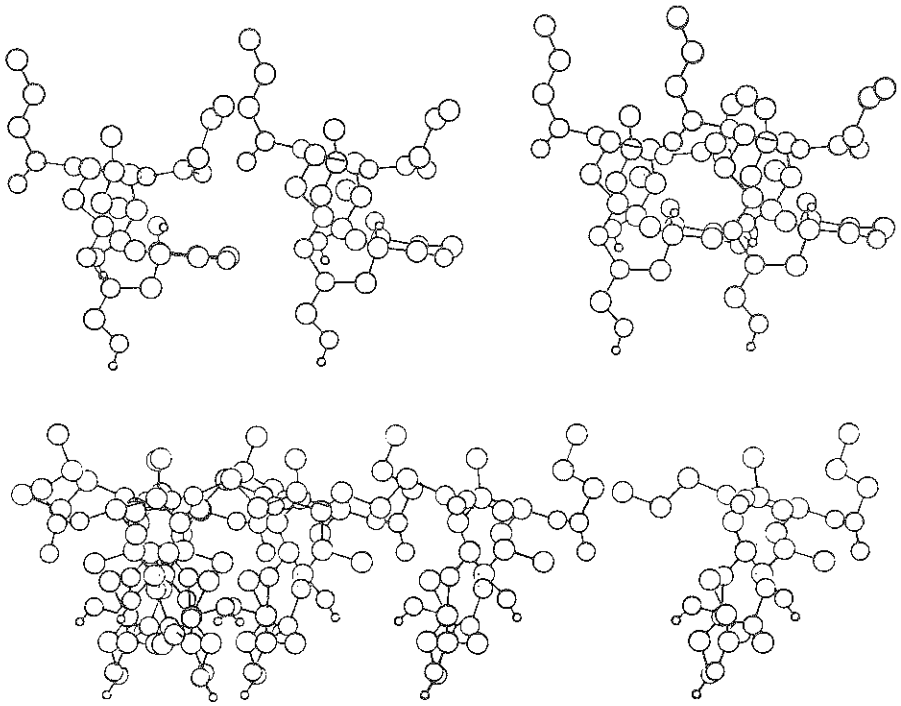

Fig. 6. Configuration of PDB monolayers established for conformers with a 56 % (upper panel) and 10 % (bottom panel) probability, respectively. Same presentation as in Fig. 1. In the upper panel, two molecules are located one behind the other.

areas (3, 5, 15). Therefore, the low theoretical area here found for the less probable conformer of 4α-PDD suggests that an increase in surface pressure may lead to a change in configuration, the energy of compression compensating for the increase in conformational energy.

In conclusion, the present data illustrate that a minor difference in the chemical structure of distinct phorbol esters, such as that distinguishing PDD from 4α-PDD, may cause considerable changes in the spatial configuration and molecular interaction at a lipid-water interface. The latter changes may well account for

differences in the tensioactive properties of these molecules (3, 5, 15), their binding to both biological and artificial membranes (9, 16) and, hence, their potency as tumor promoter (17).

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