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CONFORMATIONAL ANALYSIS OF PHORBOL ESTERS AT A SIMULATED MEMBRANE/WATER INTERFACE

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The conformation at a simulated membrane/water interface of four distinct phorbol esters, selected for their vastly different tumor-promoting potency, was predicted by a modification of the usual computing approach for the conformational analysis of macromolecules. In the modified procedure, the transfer energy of each part of the molecule in either a hydrophobic or hydrophilic domain was taken into account in order to define the orientation of the molecule at the simulated interface. The results of this study are compatible with known tensioactive properties of these phorbol esters, and may help to explain differences in their biological potency by the relative facility of their insertion in lipid bilayers.

1. Introduction

Certain phorbol esters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and phorbol 12,13-didecanoate (PDD) are potent tumor promoters. Phorbol 12,13-dibutyrate (PDB) is a weak cocarcinogenic agent, and 4α-phorbol 12,13-didecanoate (4α-PDD), the isomer of PDD, is biologically inactive (for a review, see refs. 1–3). The relative biological potency of distinct phorbol esters as tumor promoters parallels their potency in affecting functional events in a number of target cells [1,2,4–10]. It was recently proposed that such target cells are equipped with receptors for phorbol esters, the relative affinity of the receptor for distinct phorbol esters accounting for their biological potency [11–14]. It is conceivable, however, that the apparent binding of phorbol esters to their receptor in fact corresponds to a partition of the molecule between the phospholipid domain of the plasma membrane and the surrounding extracellular fluid. The idea that the biological response to phorbol esters is primarily attributable to their insertion in the phospholipid domain of target cells is compatible with several observations obtained in artificial model membranes. For instance, phorbol esters interfere with the process of Ca transport by carboxylic ionophores embedded in liposomal membranes [5,15–17]. The phorbol esters also interact with phospholipids in artificial monolayers or bilayers [18,19]. Under suitable conditions, the response to distinct phorbol esters in these artificial models parallels their biological potency [5,19]. The latter finding raises the idea that the biological potency of phorbol esters may be somehow related to the modalities of their insertion between and interaction with membrane lipids [20]. Since the latter modalities may themselves depend on the structural configuration and orientation of the phorbol ester molecules, we have analyzed in the present work the conformation of the phorbol esters at a simulated membrane/water interface.

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2. Methods

The method used for the conformational analysis of each of the four phorbol esters (TPA, PDD, 4α-PDD, PDB) is based on a strategy described elsewhere [21] and currently used for studying the conformation of polypeptides [21–24] and other molecules [25–28]. In this method, the total conformational energy is empirically calculated as the sum of the contributions resulting from the Van der Waals interaction, the torsional potential and the electrostatic interaction. The equations and parameters used for such a purpose are described extensively elsewhere [21,28,29]. The electrostatic interaction was calculated for a dielectric constant of 16, a value intermediate to those currently used for the aqueous and hydrophobic phases at a simulated interface [25–26] and indeed close to that found in the vicinity of polar heads [30]. The values used for valence angles, boundary lengths and atomic charges were those currently used in conformational analysis [21,31].

In a first systematic study, the five to eight first torsional angles of the hydrocarbon chains underwent successive increments of 60° each, yielding 6^5 to 6^8 different conformations derived from an all-*trans* conformer, taken arbitrarily as the initial configuration. A torsional angle around a given j bond is taken as positive when the distal $(j+1)$ bond rotates clockwise relative to the proximal $(j-1)$ bond [32]. The probability of existence of each conformer was calculated from the following equation

$$p = e^{-E_j/RT} / \sum_{i=1}^{n} e^{-E_i/RT}$$

with $T = 25$°C, and E_j and E_i corresponding to the internal energy of the conformer under consideration and each selected conformer, respectively. Incidentally, since long acyl chains are not very flexible in condensed states (e.g., in model membranes, see refs. 33 and 34), the effect of entropy was considered as negligible and, hence, the selection of conformers was based on their energy rather than free energy.

The conformations derived from the first systematic study and yielding a low internal energy, i.e., those with a statistical weight of at least 5%, were then submitted to a second analysis, using a simplex minimization procedure [35] in order to reduce further their total energy. All torsional angles were examined in such an analysis. The reflexion step in the minimization procedure involved variations of rotational angles from −30 to +30°, with a precision of 10°.

In a last step of the analytical procedure, the hydrophobic and hydrophilic gravity centers of selected conformers were established taking into account the transfer energy [25,36] for each part of the molecule. The values for the transfer energies used here (table 1) are identical to those determined experimentally by numerous authors and summarized elsewhere [37]. The hydrophilic center (C_w) is defined by the following equation:

$$C_w = \sum_{i=1}^{n} \left[E^+ \text{ transfer}_i \left(x_i^2 + y_i^2 + z_i^2 \right)^{1/2} \right] / \sum_{i=1}^{n} E^+ \text{ transfer}_i$$

in which x_i, y_i and z_i are the coordinates of the ith atom. The hydrophobic gravity center located in the hydrocarbon domain (C_{HC}) is defined by the same equation, except that the negative transfer energies are taken into account [36]. The orientation of the molecule at the postulated interface was then established with the axis joining the hydrophilic and hydrophobic gravity centers parallel to the Z-axis, which is itself perpendicular to the interface plane.

Calculations were made on a CDC-6600 Computer coupled to a Benson drawing table (Brussels Free University, Computing Centre, Brussels).

Table 1

Energy (in cal) of transfer into a hydrophobic domain [a]

$-\overset{\vert}{\underset{\vert}{C}}-OH$	833	$>C<$ [b]	−853
$-\overset{\Vert}{C}-O-$	4260	one degree of unsaturation	−1503
$>C=O$	2574	two degrees of unsaturation	−903

[a] Derived from ref. 37.

[b] Refers to all carbon atoms ($>C<$, $-CH=$, $-CH_2-$ and $-CH_3$)

3. Results

3.1. Phorbol backbone

In our conformational analysis of TPA, PDD or PDB, the phorbol backbone was designed, in the light of a previous study [38], with 4-OH *cis*, 8-H *cis*, 9-OH *trans* ,10-H *trans*, 11-CH_3 *trans*, 12-OH *cis* and 13-OH *trans* (fig. 1). The analysis could have been made on the mirror image, without affecting the final results. Due to the presence of a three-carbon cycle (C_{13}-C_{14}-C_{15}), the six carbon cycle (C_8-C_9-C_{11}-C_{12}-C_{13}-C_{14}) was set in the boat form with the bonds C_9-C_{11} and C_{13}-C_{14} parallel to each other. Because of the double bond between C_6 and C_7, the bonds from C_5 to C_8 were kept coplanar. The five-carbon cycle (C_1-C_2-C_3-C_4-C_{10}) was also planar.

In the phorbol backbone of 4α-PDD, as distinct from the other phorbol esters, 4-OH is *trans*. This single difference affected markedly the conformational organization of the phorbol backbone. Indeed, both the orientation of the five-carbon planar cycle and the conformation of the seven-carbon ring depended strongly on the position of OH on C_4.

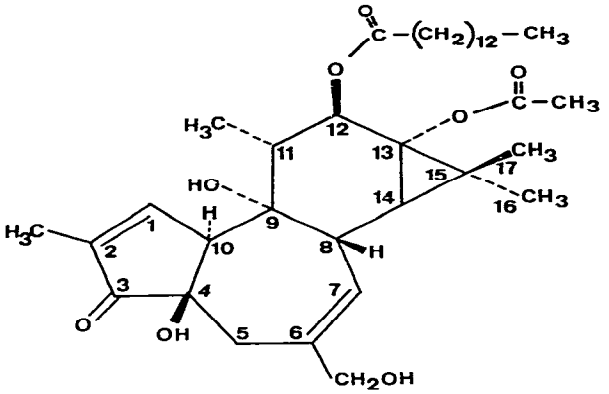

Fig. 1. Chemical formula and configuration of the phorbol backbone with myristoyl and acetyl chains on C_{12} and C_{13}. This chart also indicates the numbering of the four ring system.

3.2. Initial systematic study

A first systematic study was performed by steps of 60° each on the four first torsional angles (bonds $C_{12(13)}$-O, O-$C_{carboxyl}$, $C_{carboxyl}$ – C_2 and C_2-C_3) of both decanoyl chains in the PDD and 4α-PDD molecules, yielding 6^8 or 1 679 616 conformers. One conformer for PDD displayed a 99% probability. Fig. 2 illustrates two projections of this conformer, taken at 90° from each other. The most probable conformer for 4α-PDD displayed a 74% probability and is illustrated in fig. 3. All other conformers for 4α-PDD presented a probability of existence below 8%. The most striking difference between the most probable conformers for PDD and 4α-PDD, respectively, consisted in the position of the two decanoyl chains which were close and parallel to each other in the PDD molecule (fig. 2) but not so in the 4α-PDD molecule (fig. 3).

A comparable systematic procedure was applied to the three first torsional angles of the myristoyl chain and the two first torsional angles of the acetyl chain in the TPA molecule, and on the three first torsional angles of both butyryl chains in the PDB molecule, yielding, respectively, 6^5 (TPA) and 6^6 (PDB) conformers. The conformers with a probability in excess of 5% are listed in table 2. Figs. 4 and 5 illustrate perpendicular projections of the most probable conformer for each of these two phorbol esters.

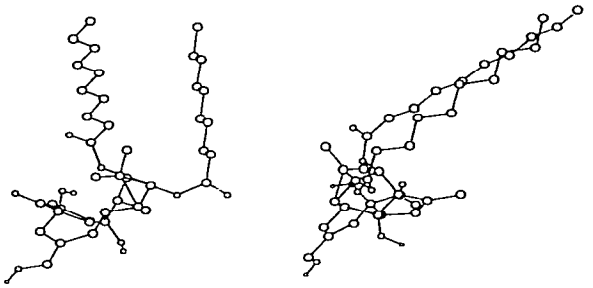

Fig. 2. View along two perpendicular axes of the most probable conformer of PDD after systematic analysis in a medium with a dielectric constant of 16.

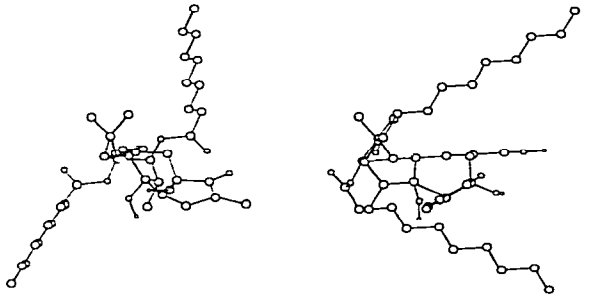

Fig. 3. Views along two perpendicular axis of the most probable conformer of 4α-PDD after systematic analysis in a medium with a dielectric constant of 16.

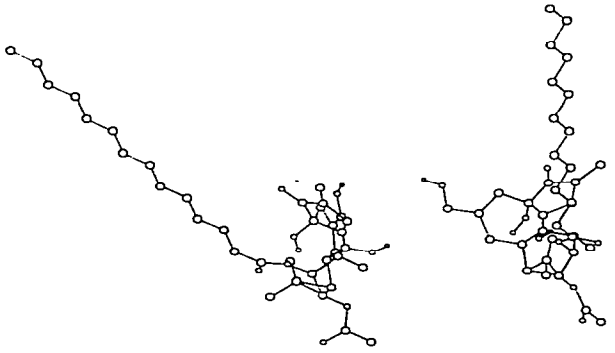

Fig. 4. Views along two perpendicula · axis of the most probable conformer of TPA after systematic analysis in a medium with a dielectric constant of 16.

3.3. Minimization procedure and reorientation

Fig. 6 illustrates the two most probable conformers for PDD and 4α-PDD, respectively, obtained after application of the simplex minimization procedure and reorientation of the molecule at the simulated membrane water interface. Likewise, fig. 7 illustrates the most probable conformers for TPA and PDB, all phorbol esters being shown in a frontal view (i.e., drawn in a plane perpendicular to the simulated interface). The value of torsional angles for these and less probable conformers, as calculated after applica-

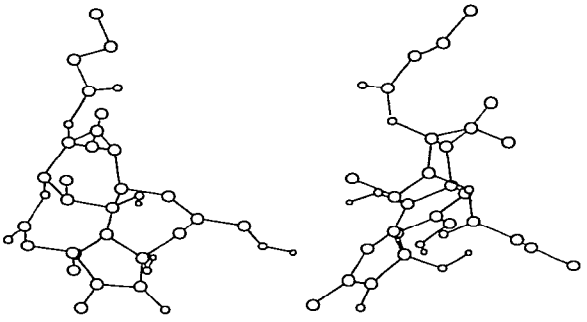

Fig. 5. Views along two perpendicular axis of the most probable conformer of PDB after systematic analysis in a medium with a dielectric constant of 16.

Table 2

Conformers of phorbol esters in equilibrium at 25°C after systematical analysis

Agents	Probability (%)	Torsional ngles of acyl chain								Energy above minimal values (kcal/mol)
		On C_{12}				On C_{13}				
		C_{12}–O	O–C_1	C_1–C_2	C_2–C_3	C_{13}–O	O–C_1	C_1–C_2	C_2–C_3	
PDD	99	240	0	120	180	120	240	240	180	–
4α-PDD	74	240	180	240	180	180	120	240	180	–
TPA	33	300	180	120		180	120			–
	27	300	180	60		180	120			0.12
	11	300	180	120		180	300			0.64
	9	300	180	60		180	300			0.76
PDB	37	300	180	120		180	300	120		–
	19	300	180	60		180	300	120		0.38
	10	300	180	120		180	300	240		0.78
	7	300	180	120		180	180	240		0.96

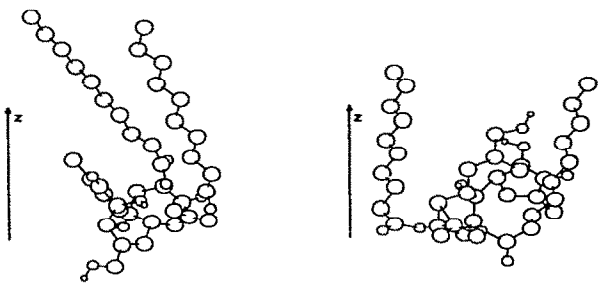

Fig. 6. Comparison between the most probable configurations of PDD (left panel, 99%) and 4α-PDD (right panel, 74%) after minimization procedure and reorientation of the molecule at the simulated interface. The molecule is shown in a frontal view, with the z-axis pointing towards the hydrophobic domain.

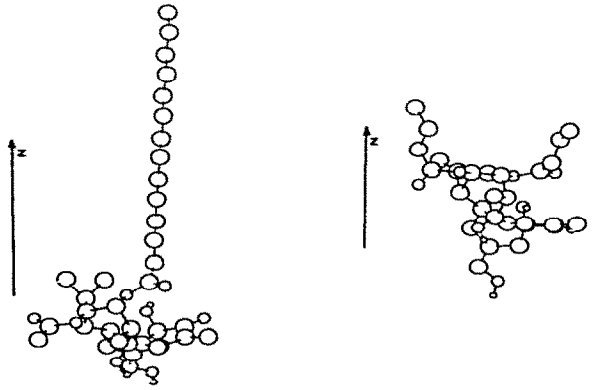

Fig. 7. Views of the most probable configurations of TPA (left panel, 60%) and of PDB (right panel, 56%) after minimization procedure and reorientation of the molecule at the simulated interface. Same presentation as in fig. 6.

tion of the simplex method, are listed in table 3, which also includes the distance between the hydrophobic and hydrophilic gravity centers of each conformer.

The orientations of TPA, PDD and PDB, respectively, at the simulated interface displayed obvious similarities. In each case, the hydrocarbon chains were oriented towards the hydrophobic domain, the hydroxymethyl group of C_6 was oriented towards the hydrophilic domain, and the overall orientation of the phorbol four-ring system was perpendicular to the plane of the interface. In the case of 4α-PDD, however, the hydroxymethyl group of C_6 was oriented towards the hydrophobic domain and the overall orientation of the four-ring system was rather parallel to the plane of the interface. Moreover, the two ester functions are now located closer to the hydrophilic phase than in the PDD molecule.

Table 3

Conformers of phorbol esters after minimization procedure

Agent	Probability (%)	Torsional angles of acyl chain								Distance between gravity centers [a] (Å)
		On C_{12}				On C_{13}				
		C_{12}–O	O–C_1	C_1–C_2	C_2–C_3	C_{13}–O	O–C_1	C_1–C_2	C_2–C_3	
PDD	99	250	0	130	188	106	310	310	169	5.25
4α-PDD	74	290	168	252	172	176	130	305	178	2.57
TPA	60 [b]	286	202	113		172	168			3.25
	20 [b]	284	202	111		178	301			
PDB	56 [b]	280	204	140		179	291	82		1.30
	10	204	124	180		173	313	274		
	7	205	114	184		175	175	295		

[a] The distance between the hydrophilic and hydrophobic gravity centers was calculated for the most probable conformers.

[b] In these cases, the minimization procedure yielded identical configurations for distinct conformers derived from the systematic analysis. For instance, the TPA conformer with a 60% probability corresponds to the two conformers with 33 and 27% probability in table 1.

4. Discussion

The present work aims at characterizing the most probable conformation of phorbol esters at a simulated membrane/water interface. The four phorbol esters under study were selected on the basis of their vastly different biological potencies [3]. The method here used for conformational analysis differs in one respect from that previously applied to the study of macromolecules at a simulated membrane/water interface [25]. Indeed, in a previous study, it was possible to locate at the interface the initial position of a given atom in the macromolecule and to complete the conformational analysis by taking into account the energy of transfer of all portions of the molecule as they moved from one environment to another, each of which was characterized by its dielectric constant. In the present case, however, there was no objective basis to ascribe a precise location to any given atom of the phorbol ester. Therefore, the orientation of the phorbol ester at the simulated membrane/water interface was determined by establishing the hydrophobic and hydrophilic gravity centers of each molecule [26,36].

The results of our study emphasize the existence of differences in the configuration of distinct phorbol esters at the simulated membrane/water interface. The most dramatic illustration of such differences consists in the configuration of PDD and 4α-PDD, respectively. Despite the fact that these two phorbol esters only differ from one another in the position of the hydroxyl group on C_4, their configurations at the interface were strikingly dissimilar. Such a dissimilarity can be explained by the fact that, in the PDD molecule, the hydroxyl group on C_4 and the ester bond on C_{12} are in close proximity favoring the approximation of and interaction between the two decanoyl chains. In the 4α-PDD molecule, the opposite position of the hydroxyl group on C_4 favors Van der Waals interaction between the acyl chain on C_{12} and the neighboring methylated five-carbon cycle (C_1-C_2-C_3-C_4-C_{10}).

The results of our conformational analysis may help in understanding differences in the biological potency of distinct phorbol esters, if the assumption is made that the insertion of these esters in a lipid bilayer is relevant to the expression of their tumor-promoting action and other biological properties. For instance, the biological inactivity of 4α-PDD could be explained, in part at least, by hindrance to its insertion in a lipid bilayer. Indeed, it is obvious from the configurations illustrated in fig. 6 that the lesser amphilic character and the greater distance between the two decanoyl chains of 4α-PDD, as distinct from PDD, both represent unfavorable attributes for insertion in a lipid bilayer. This view is reinforced by experimental findings indicating that PDD and 4α-PDD, respectively, display different surface properties in terms of both their tensioactive behavior in pure monolayers examined at low surface pressure [18] and their repulsive interaction with phospholipids [19]. Supportive energy, e.g., compression, is required to achieve comparable molecular areas for PDD and 4α-PDD, respectively [18].

In our study, TPA, which is biologically somewhat more potent than PDD, appeared less amphiphilic than PDD, this difference being attributable to the presence of only one long acyl chain in TPA. However, the presence of this single chain orientated towards the lipophilic domain may facilitate the insertion of TPA along the acyl chains of endogenous phospholipids. In the same perspective, the more globular conformation of PDB could account for the much lower biological potency of this phorbol ester relative to that of TPA.

In conclusion, the conformational analysis of distinct phorbol esters at a simulated membrane/water interface may explain differences in the penetration of these molecules in the phospholipid domain of living membranes and, hence, could account for their vastly different biological potencies.

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References

1 P.M. Blumberg, CRC Crit. Rev. Toxicol. 8 (1980) 153.
2 P.M. Blumberg, CRC Crit. Rev. Toxicol. 9 (1981) 199.
3 R.K. Boutwell, CRC Crit. Rev. Toxicol. 2 (1974) 419.
4 M. Castagna, C. Rochette-Egly, C. Rosenfeld and Z. Mishal, FEBS Lett. 100 (1979) 62.
5 M. Deleers, M. Castagna and W.J. Malaisse, Cancer Lett. 14 (1981) 109.
6 M.B. Fisher, M. Flamm, D. Schachter and I.B. Weinstein, Biochem. Biophys. Res. Commun. 86 (1979) 1063.
7 R.I. Grove and S.D. Schimmel, Biochem. Biophys. Res. Commun. 102 (1981) 158.
8 W.J. Malaisse, A. Sener, A. Herchuelz, A.R. Carpinelli, P. Poloczek, J. Winand and M. Castagna, Cancer Res. 40 (1980) 3827.
9 J. Moroney, A. Smith, L.D. Tomei and C.E. Wenner, J. Cell. Physiol. 99 (1978) 287.
10 S.D. Schimmel and T. Hallam, Biochem. Biophys. Res. Commun. 92 (1980) 624.
11 P.E. Driedger and P.M. Blumberg, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 567.
12 C.L. Ashendel and R.K. Boutwell, Biochem. Biophys. Res. Commun. 99 (1981) 543.
13 V. Solanki and T.J. Slaga, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 2549.
14 A.D. Horowitz, E. Greenebaum and I.B. Weinstein, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 2315.
15 M. Deleers, F. Defrise-Quertain, J.M. Ruysschaert and W.J. Malaisse, Res. Commun. Chem. Pathol. Pharmacol. 34 (1981) 423.
16 M. Deleers and W.J. Malaisse, Chem. Phys. Lipids 31 (1982) 1.
17 M. Castagna, M. Deleers and W.J. Malaisse, Carcinogenesis, a comprehensive survey (Raven Press, New York, 1982) vol. 7, p. 555.
18 K. Jacobson, C.E. Wenner, G. Kemp and D. Papahadjopoulos, Cancer Res. 35 (1975) 2991.
19 M. Deleers, J.M. Ruysschaert and W.J. Malaisse, Chem.-Biol. Interact. 42 (1982) 271.
20 M. Deleers and W.J. Malaisse, Cancer Lett. 17 (1982) 135.
21 E. Ralston and J.L. De Coen, J. Mol. Biol. 83 (1974) 393.
22 E. Ralston, J.L. De Coen and R. Walter, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 1142.
23 J.L. De Coen, C. Humblet and M.H.J. Koch, FEBS Lett. 73 (1977) 38.
24 J.L. De Coen and E. Ralston, Biopolymers 16 (1977) 1929.
25 R. Brasseur, E. Goormaghtigh and J.M. Ruysschaert, Biochem. Biophys. Res. Commun. 103 (1981) 301.
26 R. Brasseur, M. Deleers, W.J. Malaisse and J.M. Ruysschaert, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2895.
27 M. Deleers, R. Brasseur, M. Gelbcke and W.J. Malaisse, J. Inorg. Biochem. 16 (1982) 215.
28 R. Brasseur and H.D. Hurwitz, J. Electroanal. Chem. (1983) in the press.
29 A.M. Liquori, Q. Rev. Biophys. 2 (1969) 65.
30 M. Shinitzky, Isr. J. Chem. 12 (1974) 879.
31 A.J. Hopfinger, Conformational properties of macromolecules (Academic Press,, New York, 1973).
32 M. Sundaralingam, Ann. N.Y. Acad. Sci. 195 (1972) 324.
33 G. Buldt, H.U. Gally, A. Seelig, J. Seelig and G. Zaccai, Nature 271 (1978) 182.
34 K. Zakrzewska, R. Lavery and B. Pullman, Int. J. Quantum Chem. 8 (1981) 161.
35 J.A. Nelder and R. Mead, Comput. J. 7 (1965) 308.
36 R. Brasseur, Ph.D. Thesis, (Free University Brussels 1981).
37 C. Tanford, The hydrophobic effects. Formation of micelles and biological membranes (John Wiley & Sons, New York, 1973).
38 E. Hecker, H. Bartsch, H. Bresh, M. Gschwendt, E. Härle, G. Kreibich, H. Kubinyi, H.U. Schairer, C.V. Szczepanski and H.W. Thielman, Tetrahedron Lett. 33 (1967) 3165.