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Conformational analysis of 6-*cis*- and 6-*trans*-leukotriene B_4–calcium complexes

(endogenous ionophore/calcium/simulated interface/simulated lipid phase)

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ABSTRACT **We present a computational description of the conformation of a pair of two isomeric molecules (6-*cis*- and 6-*trans*-leukotriene B_4) forming a complex with one calcium ion. Our theoretical prediction of the membrane–water interface conformation and of the bulk lipid phase conformation of the two different isomeric complexes are in excellent agreement with experimental data on the leukotriene-mediated calcium ionophoresis in liposomes. The two isomers lead to vastly different conformations in the presence of Ca^{2+}, and the most probable conformation of the permeant species shows a globular conformation able to cross a lipid membrane.**

A number of membrane lipids contain in position 2 esterified arachidonic acid, which when released is the precursor of important signal molecules including, among others, leukotrienes. After stimulation of secretory cells, the turnover of membrane phospholipids and the breakdown of phosphatidyl inositol lead to the formation of phosphatidic acid and the release and oxygenation of arachidonic acid. The so-called "phosphatidyl inositol" response is accompanied by an enhancement of calcium flux (1–5). The ionophoretic capacity of phosphatidic acid and other anionic phospholipids (6) and of prostaglandins (7, 8) has already been tested in Pressman cells (9, 10). It was suggested that they act as calcium ionophores in membranes. The calcium ionophoresis by lipid soluble plasma membrane extracts of secretory cells has also been described in both the Pressman cell (11) and in liposomes (12). Recently, several cyclooxygenase and lipoxygenase products have been examined for their calcium ionophoretic properties in liposome membranes (13–15). It has been clearly demonstrated that 6-*cis*-leukotriene B_4, unlike 6-*trans*-leukotriene B_4 and cyclooxygenase products, is able to act as a calcium ionophore (14–15).

To obtain information on the ionophoretic behavior of these agents at the model membrane level, we have studied the conformation of two molecules of (5*S*,12*R*)-dihydroxy-6-*cis*,8,10-*trans*,14-*cis*-icosatetraenoic acid bound to one calcium [(6-*cis*$LTB_4)_2$–Ca] and of two molecules of (5*S*,12*R*)-dihydroxy-6,8,10-*trans*,14-*cis*-icosatetraenoic acid (16) bound to one calcium ion [(6-*trans*-$LTB_4)_2$–Ca] at a simulated lipid–water interface and in hydrophobic medium. We have analyzed complexes with a 2:1 stoichiometry, because monocarboxylic ionophores such as A23187 have this stoichiometry (10, 17).

METHODS

The method used for the theoretical conformational analysis of the two different Ca complexes is based on a semi-empirical method described elsewhere (18–21). Briefly, the total conformational energy that represents the sum of the contributions resulting from the Van der Waals interactions, the torsional potential, and the electrostatic interactions is calculated for a large number of conformations in a systematic analysis bearing on all angles. The conformations yielding the lowest internal energy were eventually submitted to the energy function minimization procedure (22) in a medium of low dielectric constant representative of the hydrophobic part of the membrane or at the simulated lipid–water interface (20–21). The values used for the valence angles, boundary lengths, atomic charges, torsional potentials, and energy values for the estimation of Van der Waals interactions are those currently used in conformational analysis (21, 23–26). Calculations were made on a CDC-Cyber 170 coupled to a Calcomp 1051 drawing table using the PLUTO drawing program (27).

RESULTS AND DISCUSSION

The molecular structure, the numbering of the torsional angles, together with the all-*trans* conformation of half of the two leukotriene complexes taken as our initial configurations are illustrated in Fig. 1. Each complex has 27 rotational angles, and if these angles were affected by systematic 60°C changes, $>1.023 \times 10^{21}$ conformations (6^{27}) could be designated. To avoid this large number of conformations, another procedure was used; systematic analysis was carried out in a stepwise manner on four different parts of the complex. A first systematic analysis was carried out on the angles labeled α_1, α_2, α_2', α_3, α_3', α_4, and α_4' yielding 279,936 conformations for each of the two complexes. The other three systematic analyses yielded successively 46,656 conformations for each of the two complexes, so that our approach generated 419,904 structures for each of the two $(LTB_4)_2$–Ca complexes.

Fig. 2 summarizes the most probable configuration (selection based on a Boltzmann statistical weight of all configurations) with their probability of existence in a "structure tree" obtained after four successive systematic analyses. Conformations with probabilities of existence <5% were discarded. Figs. 3 and 4 illustrate the conformations respective to the right part of Fig. 2 obtained with 6-*trans*-LTB_4 and with 6-*cis*-LTB_4. It is obvious that the 6-*cis*-LTB_4–Ca complex adopts a wide variety of conformations as compared to the 6-*trans*-LTB_4–Ca complex, even though the sequential conformational analysis was conducted in the same manner for both molecules. Fig. 5 shows the most probable conformation of the 6-*cis*-LTB_4–Ca complex after application of the energy minimization procedure to the most probable conformer of the plane symmetry type (denoted by a star in Fig. 2). It shows that the 6-*cis*-LTB_4–Ca complex adopts a weak-

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Abbreviations: 6-*cis*-LTB_4, 6-*cis*-leukotriene B_4 [(5*S*,12*R*)-dihydroxy-6-*cis*,8,10-*trans*,14-*cis*-icosatetraenoic acid]; 6-*trans*-LTB_4 [(5*S*,12*R*)-dihydroxy-6,8,10-*trans*,14-*cis*-icosatetraenoic acid].

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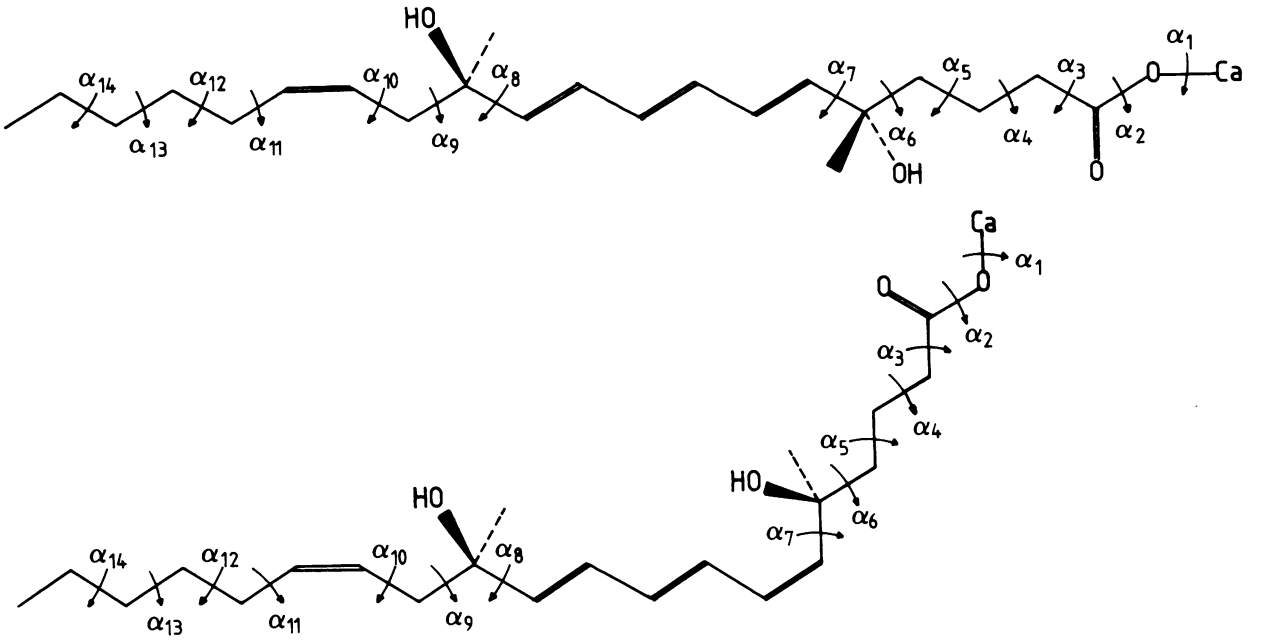

FIG. 1. Initial all-*trans* conformation of half of 6-*cis*-, and 6-*trans*-LTB_4 showing the numbering of the torsional angles.

ly oriented position at the simulated interface, and a quasi-globular shape in the medium representative of the lipid part of the membrane. This indicates that the complex molecule may assume a suitable configuration for the insertion of a Ca^{2+} ion in a cryptic hydrophilic cavity, screening the Ca^{2+} ion from the hydrophobic medium. This arrangement appears quite favorable for the transport of Ca^{2+} by 6-*cis*-LTB_4. On the other hand, 6-*trans*-LTB_4–Ca complexes (Fig. 6) adopt extended configurations in both hydrophobic medium and simulated membrane–water interface, so that the conformations with greater distances between the hydrophobic and hydrophilic centers (20) may not easily cross the membrane.

Moreover, the globular conformation of the 6-*cis*-LTB_4–Ca complex could also be the favorable structure to interact with membrane receptors, because the mechanism by which LTB_4 aided by Ca inserts in biological membranes may subsequently cause its interaction with receptors.

It could be argued that our study does not show the progressive transformation of the complex from the simulated interface into a bulk lipid phase structure. Unfortunately, this could only be obtained for small ionophoretic complexes with a relatively low number of torsional angles (28) because of the computing time. It should be noted, however, that the amphiphilic nature of the LTB_4 with its carboxylic moiety provokes a strong Ca^{2+} adsorption to membranes. This may be sufficient to interact with a second LTB_4 molecule to allow the *trans* conformation after the disappearance of electrostatic charge.

Finally, the formation of hybrid structures in membranes, like those formed by other calcium ionophores (29, 30), may also account for the transport of Ca^{2+} by this type of endogenous ionophore. Indeed, the formation of hybrid complexes between one leukotriene, one Ca ion, and another iono-

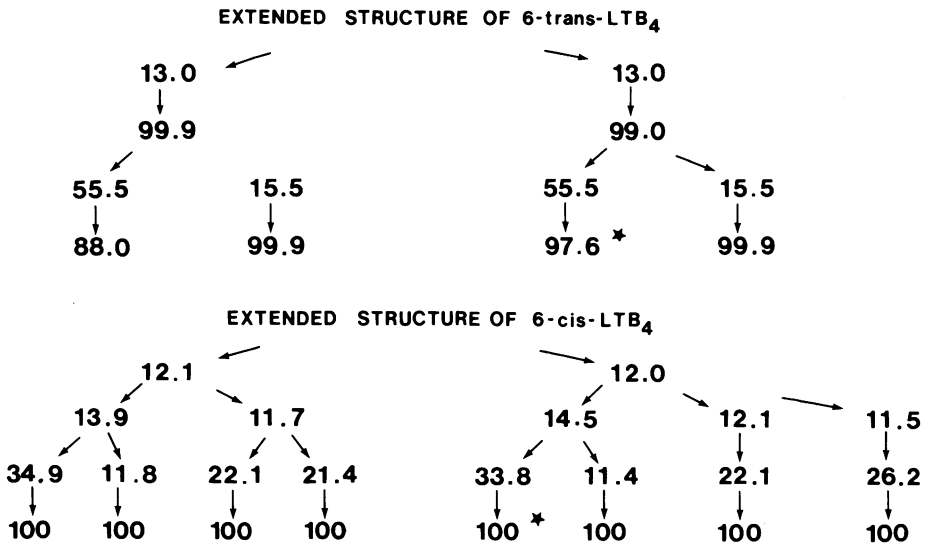

FIG. 2. Structure tree of 6-*trans*-LTB_4 and 6-*cis*-LTB_4. Structure tree was obtained after four successive conformational analyses bearing on rotational angles α_1 to α'_4 (279,936 conformations) leading to an axial symmetry (left branch) or to a point symmetry (right branch) for the first line. The three other successive conformational analyses were made on angles α_5 to α'_7, α_8 to α'_{10}, and α_{11} to α'_{13}. Right branches of each complex are shown in Figs. 3 and 4. Conformations with probabilities of existence (Boltzman) <5% were rejected.

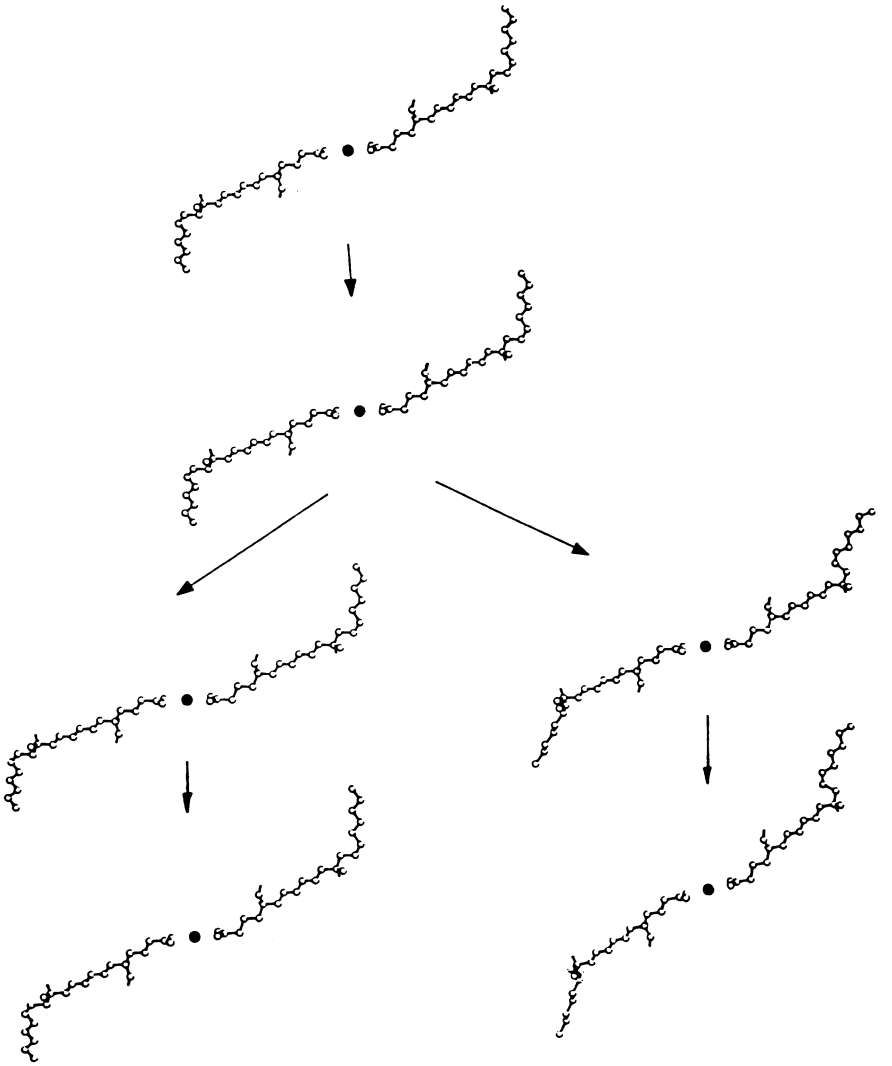

FIG. 3. Views of the most probable conformations of 6-*trans*-LTB_4 (right branch of the structure tree in Fig. 2).

phore-like phosphatidic acid or phosphatidyl serine (5, 14) could perhaps display greater ionophoretic capacity than the homologous complex. Such complexes appear conceivable because the probability of association of two identical ionophoretic molecules is low (31).

In conclusion, we have attempted to show in this study that conformational analysis performed using the same procedure on two isomeric molecules may lead to considerable differences in the final theoretical Ca complexes. The presence of the 6-*cis*–*trans* isomerization indeed accounts for different biological activities (32, 33), for different ionophoretic properties in liposomes (15, 33), and for two vastly different Ca complexes.

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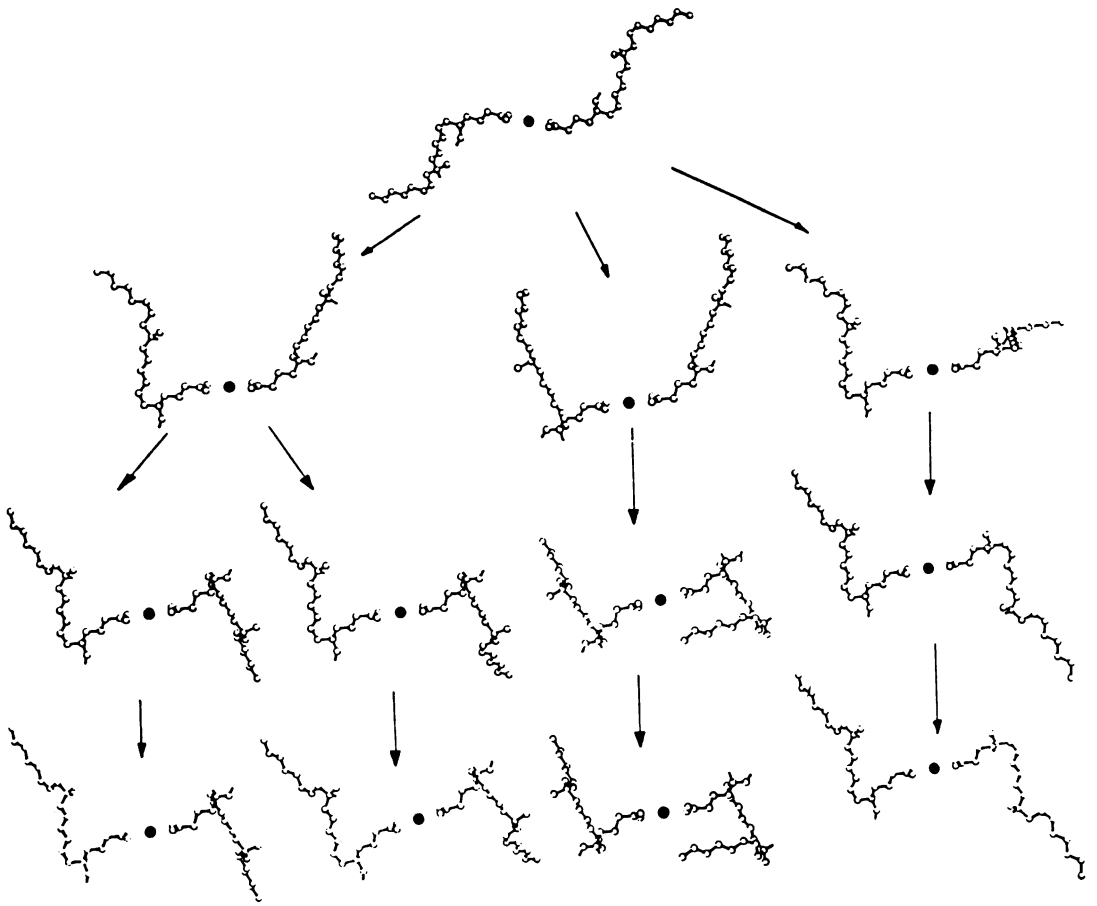

FIG. 4. Views of the most probable conformations of 6-*cis*-LTB_4 (right branch of the structure tree in Fig. 2).

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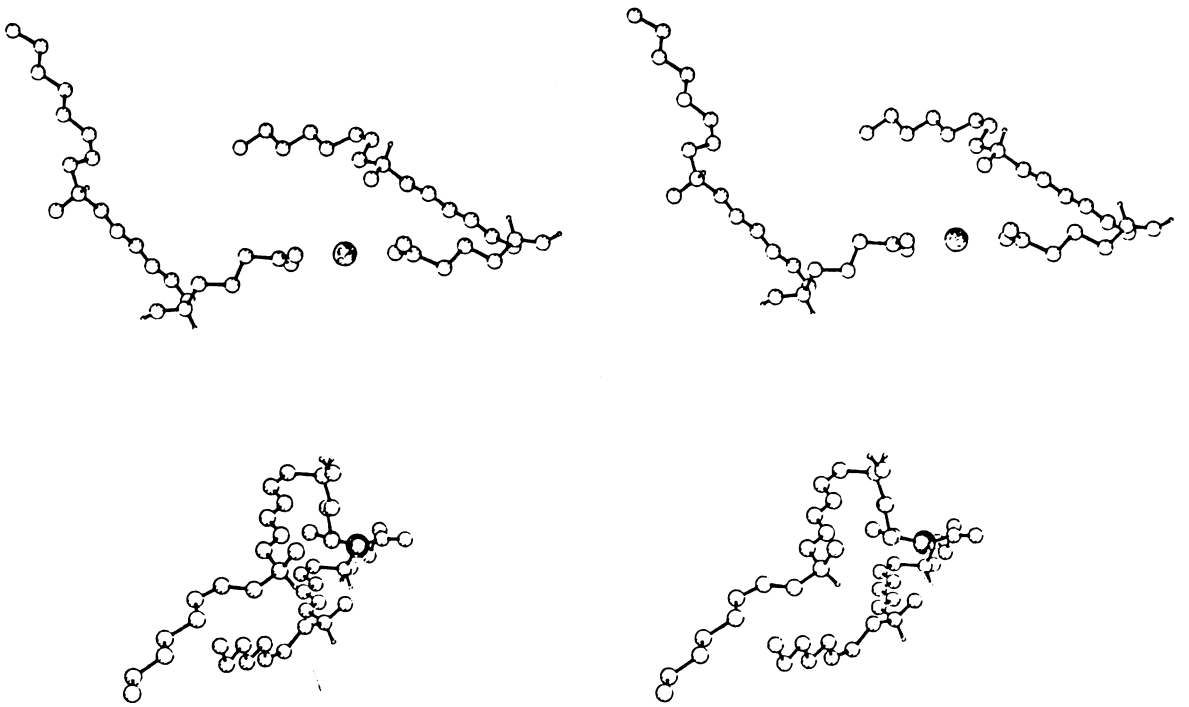

FIG. 5. Stereoscopic views of the most probable conformation of 6-*cis*-LTB_4 after application of the simplex minimization procedure, showing the interfacial complex (*Upper*) and the bulk lipid phase complex (*Lower*).

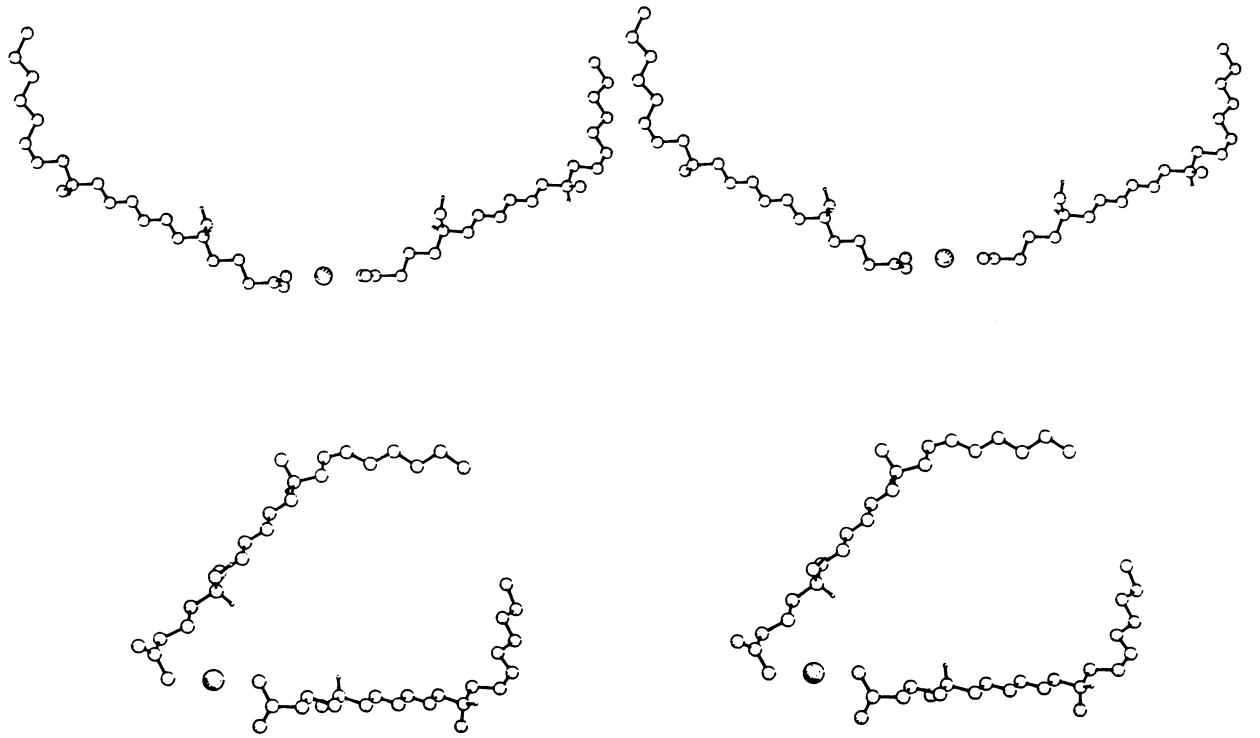

FIG. 6. Stereoscopic views of the most probable conformation of 6-*trans*-LTB_4 after application of the simplex minimization procedure, showing the interfacial complex (*Upper*) and the bulk lipid phase complex (*Lower*).