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Conformational Energy Calculations on Valinomycin

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by

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Abstract

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The antibiotic action of the ionophore valinomycin is a result of its ability to preferentially bind potassium ions and transport them across cellular membranes. Complexation is solely due to the molecular structure of this cyclic depsipeptide. In solution, the molecule exists in different conformers depending upon solvent polarity. Exact knowledge as to the structure of valinomycin in the aqueous and lipid cellular layers is thus difficult to determine. Many spectral studies of valinomycin have been undertaken utilizing a variety of instrumental methods and conformations existing in different solvents have been proposed. We have performed energy minimization procedures on these structures. Conformations were generated both with and without a symmetry condition. Utilization of these mathematical models resulted in structures which agree with those described in the literature. Thus, these calculations may be combined with solution studies to determine the exact structure of valinomycin before, during and after ion transport.

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Introduction

Valinomycin is from the class of biological compounds known as ionophores or complexones. Ionophores are compounds that form lipid soluble complexes with polar cations. This complexing ability accounts for their ability to transport ions across biological membranes. Complexation, and thus the ionophore's biological activity, is dependant upon their conformation.

The ionophores are atypical proteins. They are not composed solely of amino acids; rather, they consist of amino- and hydroxy-acid residues linked by amide and ester bonds (see figure 1).

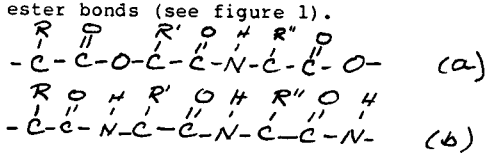

Figure 1. Depsipeptide (a) compared to typical peptide (b).

Although it may appear that ionophores function somewhat as enzymes; in fact, they differ greatly from this protein class. Enzymes are large macromolecules whose activity is usually dependant upon an active site which is stereospecific for a given reaction. Usually, if an amino-acid residue far from the active site is changed, there is little effect on enzymatic activity. This is

not the case for ionophores. Changing a residue in these compounds greatly reduces their biological action. Additionally, the kinetics and diffusion rates of ionophores across lipid membranes result in turnover numbers with values in thousands per second (1). These high values are greater than the turnover numbers of most enzymes. Finally, ionophores contain both D and L residues, whereas most biological compounds,including enzymes, are only composed of L.

The complexation ability of ionophores such as valinomycin arises from their conformation. These cyclic molecules can assume a specific conformation which focuses the carbonyl oxygens about a ring. These interior, electronegative atoms then "solvate" the cation (1). The exterior of the molecule contains nonpolar hydrocarbon side chains and the complex becomes lipid-soluble. The three-dimensional cavity in valinomycin is a perfect fit for potassium ions. Thus valinomycin shows a 10,000:1 preference for K^+ (ionic radius of 1.33A) over Na^+ (ionic radius of 0.95A) (1). The preciseness of this cavity accounts for the fact that changing the structure generally results in compounds less capable of transporting potassium ions.

The exact mechanism for the transport of ions by neutral complexones such as valinomycin is uncertain. It is known that transport in the membrane occurs in the

complexed form. The formation of this complex is governed by the reaction:

$$V + K^+ \rightleftharpoons VK^+$$

which can occur on the membrane surface or in the premembrane layer (2). These two possibilities are known as the small and large carrousel, respectively (see figure 2).

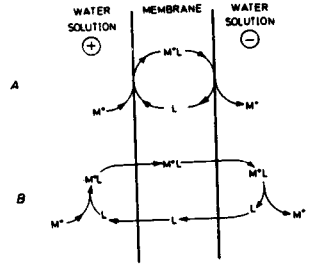

Figure 2. Possible mechanisms for the electrophoretic transport of cations coupled with circulation of the neutral carrier molecules. Complex formation and breakdown are taking place: *A*, at the membrane—aqueous solution interfaces ("small carrousel"), or *B*, in the non-stirred pre-membrane layers of the aqueous solution ("big carrousel"). (8)

Additionally, it is unknown whether a single molecule carries each ion or whether there is a relay mechanism involving two or more molecules within the membrane (3). It is known that the concentration of ions on each side of the membrane controls the rate and overall direction of transport.

The study of the ionophores is of great importance to biological investigators. Ionophores not only can provide insight into the molecular basis of cation transport, but also can be used to study the relationship and dependance of metabolism and ion transport (1). Ionophores are viewed as model biological carriers.

Valinomycin, originally isolated from bacterial broth, is a highly selective and efficient transporter of potassium ions. It has been hypothesized that similar compounds exist in ion-transporting membranes of higher organisms and researchers have examined cellular membranes in attempts to prove or disprove this theory (1).

The ability of valinomycin to preferentially bind and transport potassium ions has resulted in its use as an ion-sensitive electrode. These electrodes can measure potassium ion concentrations of 10^{-5} M in a 0.1 M sodium chloride solution (2). Valinomycin electrodes are used for determining potassium concentrations in soils, sea water, and biological fluids -- specifically intracellularly.

Valinomycin has also been studied by researchers in a variety of disciplines. Molecular biologists use this compound to study the effect of ion concentration and ion gradient on active transport as well as on metabolic and catabolic processes (2). Theoretically oriented investigators are interested in determining its structure and conformation in order to clearly elucidate the relationship between conformation and complexation.

Valinomycin consists of three identical tetradepsipeptide fragments: (D-Val-L-Lac-L-Val-D-Hyiv) (see figure 3).

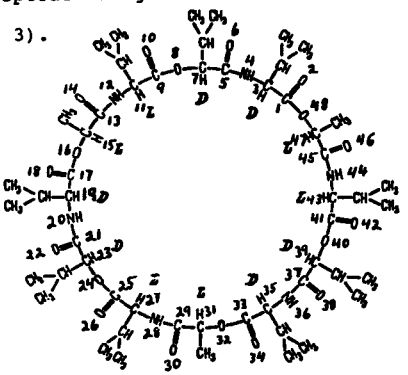

Figure 3.
The sequence of valinomycin. Only the ring and carbonyl atoms are numbered.

The cyclic nature of the molecule, combined with the three repeating segments, lend it the potential to have a symmetric conformation. In a molecule with C_n symmetry, rotation about the symmetric axis leads to the starting conformation. The following relationship also holds:

$$W_{i+km} = W_i \quad (i=1,2,\ldots m;\ k=1,2,\ldots n-1)$$

where n is the number that describes the symmetry and m is the number of dihedral angles in a symmetry unit. When a molecule possesses C_n symmetry the first and the (m+1)th local coordinate systems are symmetrically related (4). Rotation by $2\pi/n$ results in the overlap of the (m+1)th coordinate system by the first. Examining the covalent structure of valinomycin, we see that rotating the molecule by 120° leads us back to the

original conformation, thus n=3 and it is possible for valinomycin to possess C_3 symmetry. Additionally, valinomycin may also possess S_6 symmetry as rotating it by 30° and then inverting the compound leads to the same starting structure (4).

There are difficulties in studying the structure of valinomycin. Although X-ray crystallography has been done on both the complexed and uncomplexed forms (5,6,7,8), the crystal form lends little to the study of complexation. It is in aqueous and lipid solutions that valinomycin functions as a biological cation carrier. Therefore, it is solution studies which must be used to determine its conformation.

A plethora of spectral studies have been undertaken to help elucidate the structure of this important ionophore (6,9,10,11). Proton NMR studies yield information regarding the backbone angles, while C NMR elucidates the side chain conformers. Additionally, infra red analysis shows intramolecular hydrogen bonds and also aids in the determination of which hydrogen bonds change with changing solvent.

The result of all these investigations is that valinomycin is known as the "first peptide molecule whose spatial structure was established" solely utilizing solution studies (3). It has been determined that in solution valinomycin exists as an equilibrium mixture of

three major forms and its specific conformation is highly solvent-dependant. Form A, predominant in nonpolar solvents, has a three-fold axis of symmetry. All the amino groups participate in intramolecular hydrogen bonding with the amide carbonyls. In solvents of medium polarity form B predominates. It retains the three more stable hydrogen bonds involving the valyl amino groups. Finally, in polar solvents valinomycin assumes form C which has no rigid structure. Rather, it is a rapid equilibrium of several interconverting forms in which all the amino groups are hydrogen bonded to the solvent (15). Additionally, as the polarity of the solvent is slowly varied, a number of additional forms have been observed. These compounds are intermediate between A, B and C with 5,4,2 or 1 internal hydrogen bonds (2).

In nonpolar solvents, valinomycin A resembles a bracelet. It is approximately 8Å in diameter and 4Å high. There are two possible arrangements of the depsipeptide chain which will yield this structure, A1 and A2. Form A1 has all the ester carbonyls oriented outward, and is therefore called the "all out" conformation. Conversely, form A2 is known as the "all in" conformation with these carbonyl groups directed inward. The latter form is calculated to have an energy of 12.8 Kcal/mole and is energetically more stable than the former with an energy of 27.6 kcal/mole (2). However, NMR evidence indicates that in fact the A2 form

is not present in nonpolar solvents due to electrostatic repulsion by the six inward electronegative oxygens (2). Thus, in nonpolar solvents it is the A1 form which predominates (see figure 4).

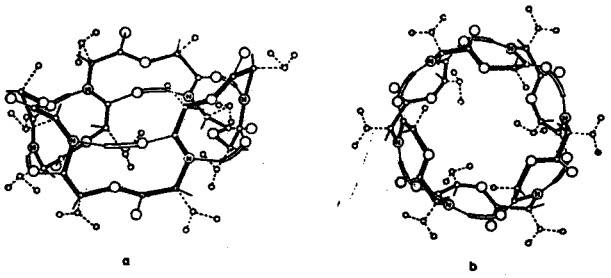

Fig. 4 Conformation of valinomycin in non-polar solvents. a, side view; b, view along the symmetry axis.(9)

Form B, stabilized by three intramolecular hydrogen bonds, is more flexible than form A. It has three-fold symmetry and acquires a "propellar" structure. (see figure 5). Interestingly, the center is hydrophobic while the surface contains the polar groups. Also, there is a low barrier to interconversion between this conformation and the A forms (11).

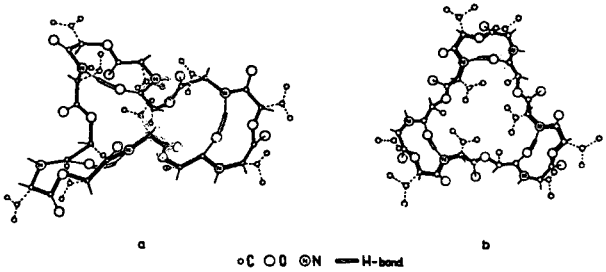

Fig. 5. Conformation of valinomycin in solvents of medium polarity. a, side view; b, view along the symmetry axis. (8)

Examination of the potassium-complexed form of valinomycin indicates that its conformation is not dependant upon solvent polarity. Once again, the molecule assumes a bracelet arrangement this time with the A2 "all in" conformation (see figure 6). This allows for the interaction of the electronegative ester carbonyls with the cation which stabilizes the electrostatic repulsion (2). Additionally, the cation is effectively shielded which prevents solvent attack and allows the surface of the complex to be lipophilic.

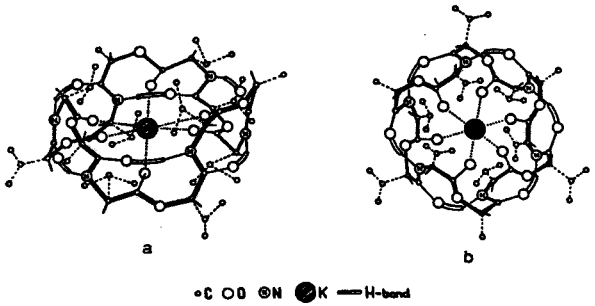

Fig. 6 Conformation of valinomycin·K^+ complex. a, side view; b, view along the symmetry axis. (9)

Comparison of the conformation of free valinomycin in nonpolar solvents (i.e. at the membrane surface) and of its complex indicate that complexation is not simple. The obvious example of this is that free valinomyicin is the A1 "all out" arrangement while the complex is the A2 "all in" form. The cation can not easily enter the cavity and be bound. Rather, it is hypothesized that the interconversion of these forms involves an intermediate; namely, form B (see figure 7) (2).

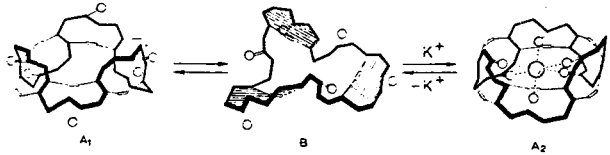

Fig. 7 Conformational equilibrium of valinomycin in the presence of potassium ions.(8)

The key to the biological activity of valinomycin is these conformational changes. The capacity to change conformation with a change in environment is the reason for valinomycin's ability to preferentially bind potassium ions, interact with a lipid membrane, and transport the ion across the barrier. Thus, it is necessary that, before any accurate determination of the mechanism of ionophore transport can be made, we must be certain as to the validity and accuracy of the proposed structures.

The conformations discussed above are based on inferences from spectral studies to yield probable angles. In order to determine how reliable these data are, conformational energy calculations can be performed. Computer programs exist which calculate the relative energies of different conformations for a given molecule. This overall energy is determined by combining electrostatic interactions, nonbonded and hydrogen bonded interactions and general torsional energies. These programs then also minimize conformational energy corresponding to local energy minima (13).

In the calculation of conformational energy, it is assumed that the bond stretching and bonding bending angles are fixed. In fact, this is not accurate; in solution a molecule does both bend and stretch. However, the error added by this assumption is small and the simplification large so that only the dihedral angles are then treated as variables (16). Additionally, the number of variables is further reduced by assuming the peptide and depsipeptide units are planar. In this way only the angles connecting two such units need be considered.

Conformational energy minimization procedures alter a starting conformation so that its energy decreases toward a minimum. In order to make maximum use of the changes that have occurred in the previous steps, drastic changes in conformation are to be avoided. However, as a

change in a few degrees in one dihedral angle can have a drastic effect on the overall conformation of the molecule, making only small, local changes is difficult. In order to result in only a local deformation in the molecule, a certain sequence of dihedral angles must be changed simultaneously. A mathematical solution to the problem of local deformation can be found by converting the coordinate systems of the molecule to a system of equations in six unknowns. Subsequent manipulations of these equations results in a single equation with only one unknown (16).

It turns out that at least seven consecutive angles must be changed simultaneously in order to deform the conformation of a molecule locally. One of these can be varied independantly while the values of other six angles are determined by the value assumed by the first (16).

In addition, the calculation of a set of dihedral angles which results in a closed ring is not facile. Many investigators (ourselves included) have relied on the chance closure of rings. Often, actual closure was obtained by imposing a ring closing potential (17). In this way, when the end groups were far apart the overall energy of the conformation artificially went up. However, this approach is generally undesirable for obvious reasons. It is advantageous to eliminate the element of chance and to begin with mathematically closed

rings.

Once again, linear algebra may be applied to the same set of equations used in the case of local deformation (ring closure turns out to be a special case of this technique). For a nonsymmetric, closed ring there are, once again, six variable dihedral angles whose values are determined by the condition of ring closure. However, for a molecule with C_n symmetry, it turns out that there are only two dependant angles in each symmetric unit (4). In the case of valinomycin, there are four residues (or eight backbone angles) in each unit. Of these eight, six will be independant while the value of the remaining two will be determined so that the ring will close.

The purpose of this paper will be to show the advantages achieved in utilizing the symmetry condition to generate conformations corresponding to exactly closed rings. Additionally, the ability of energy calculations to augment spectral studies in order to determine unique conformations in solution will be demonstrated.

Experimental

Energy minimizations and calculations were performed on the literature conformations of valinomycin. Computations were performed utilizing the FORTRAN program EMIN (18) and the capabilities of a Burroughs B6805. Graphical pictures were later obtained using the FORTRAN program NAMOD (19) linked with the IGL library off a VAX 11/780 and drawn using a Hewlett Packard 7580A plotter.

The backbone and side-chain angles as given in the literature were used (see Table 1). However, when a complete set of side-chain angles for L-Val, D-HyIv, and D-Val were not provided, the procedure of Krishna, et. al. (14) was followed: For "A" and "C" conformers, χ^1 and χ^3 were set equal to 180° and χ^2 to 60°, while for "B" conformations, χ^1 was set to 180° with χ^2 and χ^3 equal to 60°. In all cases, we set χ of L-Lac to be 60°. Finally, unless a defined value was given, ω was assumed to be 180°. Amino and carboxyl groups were used as the N and C termini respectively.

Strategy A

Minimization was carried out in three parts. First, the backbone angles were varied while the side-chain angles were held constant.

Table: Starting Torsional Angles of the Literature Valinomycin Conformation

Reference	L-Val						D-Hylv					
Crystal	ϕ	ψ	ω	χ'	χ^2	χ^3	ϕ	ψ	ω	χ'	χ^2	χ^3
Neupert-Laves	-58.3	130.4	175.1	-64.3	60	180	79.4	3.3	-179.4	-62.5	60	180
	-60.0	132.7	176.6	-59.9	60	180	86.4	-5.1	-170.1	-58.2	60	180
	-57.4	132.9	172.8	-61.0	60	180	79.4	8.0	-179.9	-66.3	60	180
Smith	-110	80	174	176	-68	180	147	-6	173	69	-58	180
	-67	132	174	174	-70	180	81	3	179	74	-50	180
	-71	130	177	178	-68	180	98	-7	177	166	-74	180
Karle	-63	129	174	179	-62	180	96	-3	-179	164	-68	180
	-108	78	176	177	-62	180	146	-11	172	160	-64	180
	-67	130	179	177	-64	180	82	3	-170	76	-49	180
A												
Krishna A	25	70.651	180	180	60	180	100.54	-20.052	180	180	60	180
Bystrov A	-80	90	180	180	60	180	120	0	180	60	60	180
Patel I	30	60	180	180	60	180	100	-60	180	180	60	180
Patel C-I	-70	110	180	180	60	180	70	30	180	180	60	180
B												
Krishna B	50	62.534	180	180	60	60	97.458	-39.999	180	180	60	60
Patel II-1	30	90	180	180	60	60	100	-60	180	180	60	60
Patel II-2	-140	-100	180	180	60	60	100	-60	180	180	60	60
Bystrov B	-85	100	180	180	60	60	120	0	180	60	60	60

Reference			D-Val				L-LaC			
Crystal	ϕ	ψ	ω	χ'	χ^2	χ^3	ϕ	ψ	ω	χ
Neupert-Laves	58.6	-132.7	-173.5	65.2	60	180	-76.2	-12.3	177.8	60
	57.2	-128.7	-176.8	63.5	60	180	-66.0	-25.1	177.5	60
	57.6	-131.2	-176.0	64.1	60	180	-73.1	-15.9	178.9	60
Smith	54	-133	173	63	-178	180	-100	13	-171	60
	105	-68	-171	58	-178	180	-165	-31	-176	60
	67	-136	179	61	-174	180	-71	-11	179	60
Karle	63	-134	-178	-178	57	180	-74	-6	174	60
	60	-135	-172	-178	68	180	-98	14	173	60
	108	-69	-172	-177	62	180	-164	23	-178	60
A										
Krishna A	-40	-69.32	180	180	60	180	-99.6	30.438	180	60
Bystrov A	90	-90	180	180	60	180	-120	0	180	60
Patel I	-40	-60	180	180	60	180	-100	60	180	60
Patel C-I	70	-110	180	180	60	180	-70	-30	180	60
B										
Krishna B	120	86.383	180	180	60	60	-58.341	118.86	180	60
Patel II-1	140	100	180	180	60	60	-100	90	180	60
Patel II-2	-40	-60	180	180	60	60	100	90	180	60
Bystrov B	110	90	180	-60	60	60	-120	120	180	60

Next, this procedure was reversed; the side-chain angles were varied while the already minimized backbone angles were held constant. Finally, a low energy conformation was arrived at by putting the previous two parts together and varying all the angles. In all three steps, minimization was assumed to be complete when the change in total energy was calculated to be less than 0.001 Kcal/mole. The dielectric constant was set equal to 2 for these preliminary calculations.

Strategy B

The second step involved incorporating the symmetry condition into the minimization procedure. EMIN was modified so that the interactions between appropriate atoms in the first and last residues were assumed to be 1→4 rather than 1→5. Ideally, the symmetry condition should have been directly incorporated into the minimization procedure. In fact, this did not occur. Rather, it was called as a subroutine after 10 minimizations.

The program proceeded as follows. The starting conformation was closed with a call to symmetry and the resulting conformation was minimized 10 times. The minimized angles from the center symmetric unit were then used to again close the ring and continue another run through the minimization routine. These angles were

chosen in the hope that since they were between two other symmetric units, and not on the ends, they would act similarly to a truly closed molecule.

Results and Discussion

Figure 8 shows examples of the starting conformations as given in the literature. It is obvious that they are often neither cyclic nor symmetric in actual conformation. In a and b there is considerable overlap of the end groups, while in c the molecule is rather linear. This descrepancy may be accounted for by the authors' utilization of bond lengths and angles which differ from ours.

Minimization without the symmetry requirement resulted in reasonable conformations. The average number of minimizations required for each conformation was approximately 250. The final energies were in the 10^2 to 10^3 Kcal/mol range. The resulting structures (see figure 9) are useful. The rings approach closure. Note that the starting conformation that was not closed originally (b) now approaches a closed ring, although there is still evidence of end group repulsion in it. Additionally, end group overlap has been diminished in the other conformation (a).

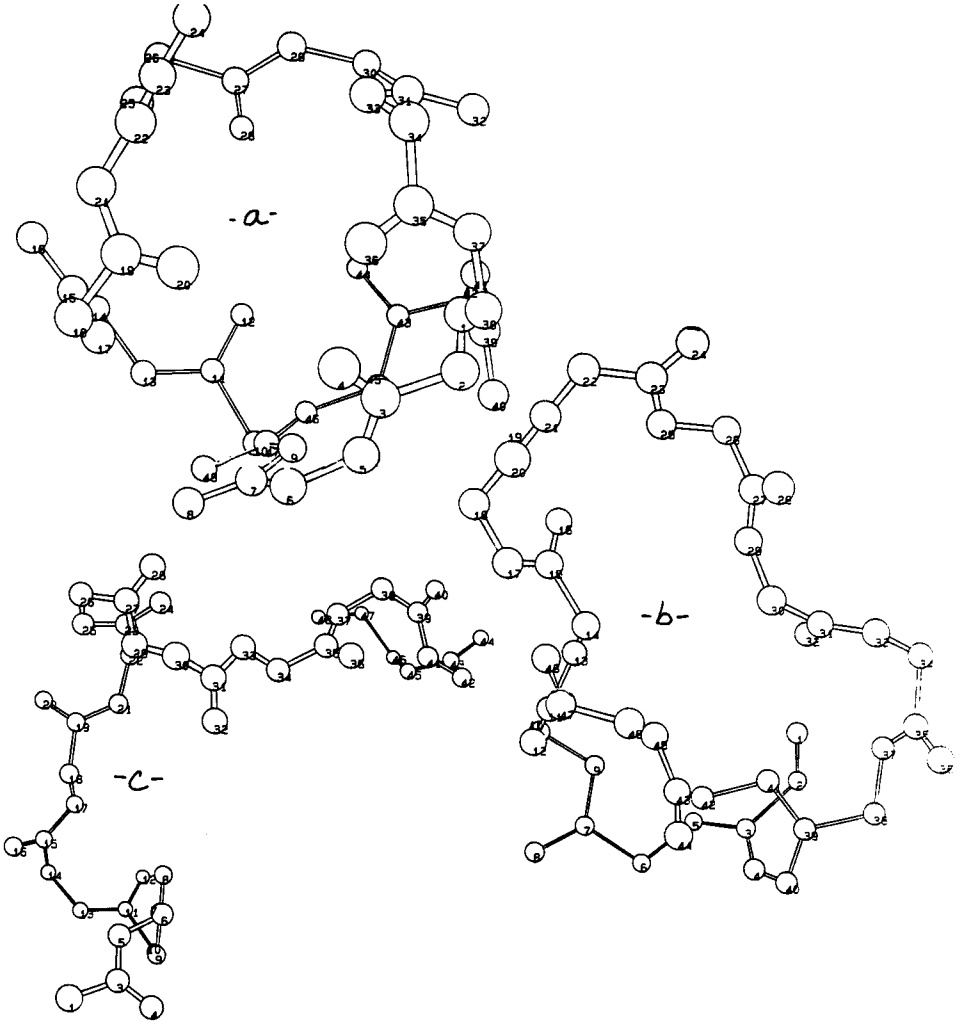

Figure 8. Starting Conformations:
a, Bystrov A; b ,Bystrov B; c, Patel II-2.

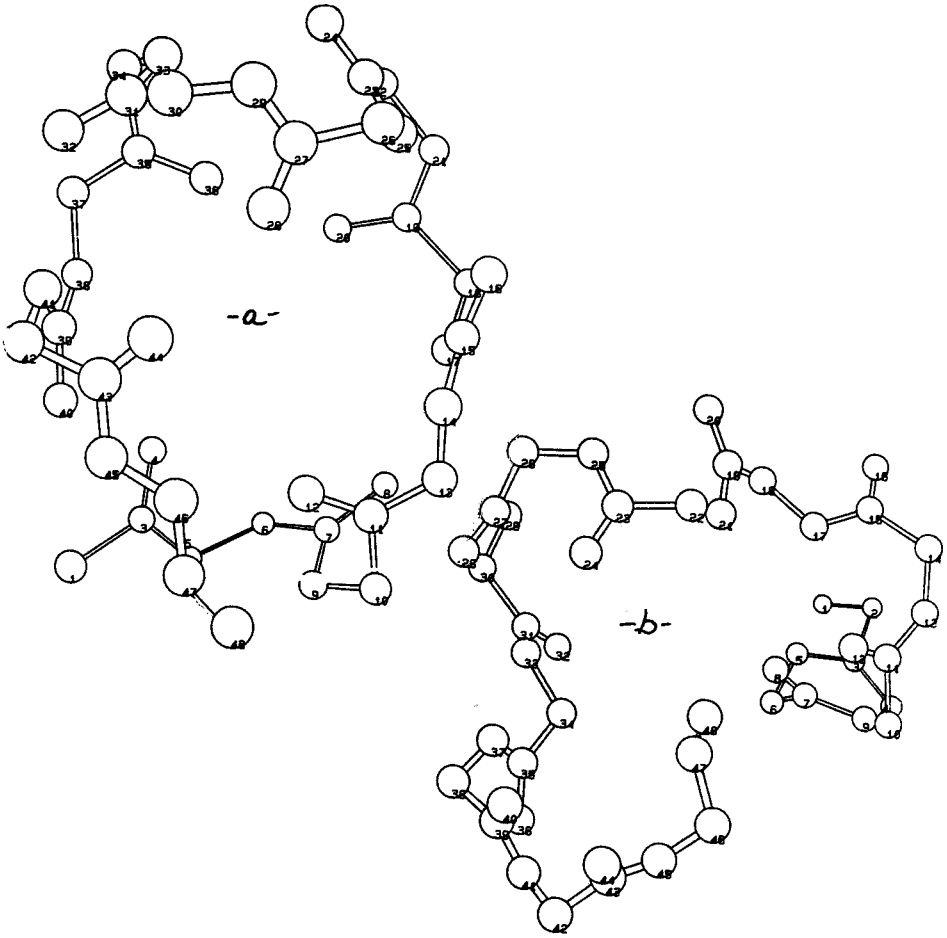

Figure 9. Minimized Without Symmetry Condition:
a, Bystrov A; b, Patel II-2.

When the symmetry condition was imposed additional problems were encountered. Often, the combination of minimization and symmetry resulted in conformations of increasing, rather than decreasing, energy. Sometimes the structures would originally decrease to a minimum and then increase. Reasonable energies were taken as those $< 10^5$ Kcal/mol. Only three conformations met this criteria (see fig. 10). However, they indicate that in spite of the present problems, this method has the potential for great success. There is no overlapping or repulsion of endgroups in any of the structures. They appear to be symmetric and (b) even agrees with the literature for what a type "B" - propellar conformation should look like.

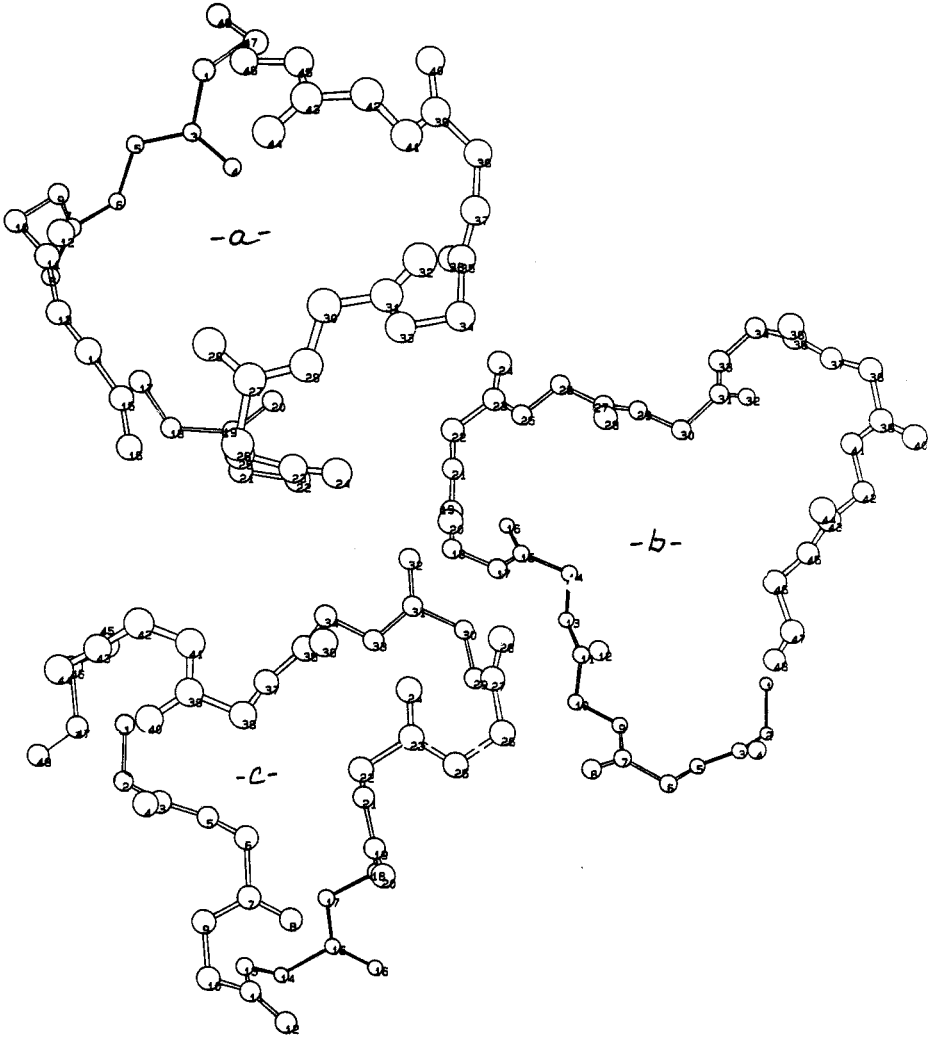

Figure 10. Minimized With Symmetry Condition: a, Bystrov A; b, Bystrov B; c, Patel II-2.

Conclusion

We have shown that utilizing a symmetry condition to generate closed structures aids in the minimization technique and may yield conformations in agreement with those predicted by NMR and solution studies. However, our goal has not been completely achieved as several problems still need to be ironed out.

First, the symmetry condition must be included and continuously maintained within the minimization procedure. In this way, the molecule will naturally minimize to cyclic conformations without endgroup repulsion or overlap. This is essential since we are dealing with a closed ring system that does not, in actuality, possess end groups. This would also eliminate the problem of deciding what set of angles should be taken as a symmetric unit; if the angles minimize to symmetric conformations all three units will have angles with the same values.

The next area that needs examination is that of choosing the two dependant angles within the symmetry condition. The symmetry subroutine now installed only works when the equation is solved in one specific manner. If, in fact, the "closest fit" is found when this has no solution, the program fails to select the correct value.

In spite of these problems, the future appears quite bright. In fact, the mathematical models can be successfully incorporated and utilized. Conformational energy calculations on cyclic molecules can be a powerful tool when coupled with solution studies to determine, specifically and uniquely, the angles in a molecule.

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