CONFORMATIONAL ANALYSIS OF A NEW CYTOCHALASIN USING MOLECULAR MECHANICS AND NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC DATA

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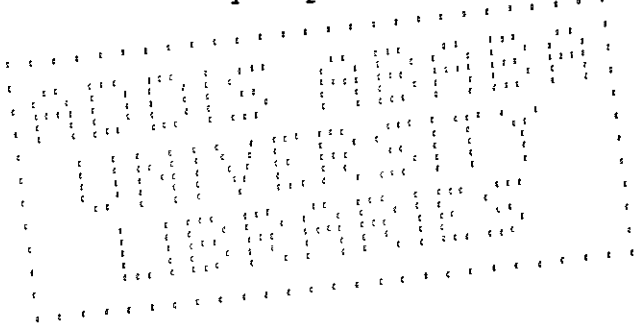


ABSTRACT

This project uses molecular mechanics techniques with the aid of NMR data to determine low-energy conformations (three-dimensional structures) of a novel cytochalasin isolated from fungus Xylaria Obovotá[1]. Although the two-dimensional structure for this substituted hydrogenated isoindole unit fused to an 11-membered ring has been postulated, no study of the three-dimensional structure has been made.

Molecular mecanics techniques, the force field equations, and conformational searching algorithms: the stochastic search (random search method) on cartesian and internal coordinates have been applied. A total of 22 conformations were generated within 10 kcal/mol to the global minimum. The calculated proton coupling constants, averaged over the first six lowest-energy conformations, were compared with the experimental values. The geometry parameters, the calculated coupling constants, the structure of the isoindole unit, the macrocycle and overall structure of the cytochalasin were also compared with similar cytochalasins whose structures were determined from x-ray and related experimental techniques. Comparison of the calculated coupling constants and the various torsion angles for this novel fungal cytochalasin revealed similarity in the conformations of the structures generated from the analysis and those determined experimentally.

1. INTRODUCTION

Chemical calculations that can predict the structures, energies, and other properties of known and unknown molecules are increasingly becoming important tools in chemical research. Experimental techniques usually provide one basic piece of information concerning a chemical compound such as structure from NMR, IR, and mass spectroscopy. However, in addition to structure and energies, the chemical calculations can also predict heat of formation, dipole moment, ionization potential, charge densities and bond order as a result of one calculation[2].

Although experimental results are often considered the best evidence, chemical calculations have some advantages. In some cases, the calculated properties have shown to be more accurate than experimental data. Measuring heat of formation of a polycyclic alkane, for instance, is a long and difficult task that requires the utmost experimental precision and sample purity. For a very large range of such alkanes, however, the most modern molecular mechanics methods allow the calculation of heat of formation that differ from the "true" values by at most 2 kcal/mol, and probably by a lot less. And the calculation delivers an optimum molecular geometry as a by-product[2].

There are two commonly employed theoretical methods for the study of the structure of molecules. These are based on quantum chemical or semiclassical models of molecular structure. In quantum chemical calculations the Schrödinger equation is

solved for a given nuclear configuration, followed by an adjustment of nuclear configuration so as to minimize the energy of the molecule. Quantum chemical methods in which all the integrals of theory, be it variational or perturbative, are exactly evaluated are referred to as ab initio methods. On the other hand, molecular mechanics methods, or force field, calculations are based on simple mechanical models of molecular structure. It is an empirical method, and is based on a large volume of experimental data. Somewhat between ab intio and molecular mechanics are the semiempirical quantum chemical methods. Like molecular mechanics methods, they use experimentally determined parameters to strive for accuracy. Like first principle ab initio methods, they are quantum mechanical in nature.

The principal aim of this work is to apply molecular mechanics techniques together with conformational searching methods to determine the global energy minimum and other low-energy conformations of novel cytochalasin obtained from fungus Xylaria Obovota[1].

The cytochalasins are a group of fungal metabolites which inhibit cell division and motility[3]. These compounds are characterized by a highly substituted isoindole system, to which is fused a macrocyclic ring. The trivial name "cytochalasin," was given because of their unexpected toxicity toward mammalian cells[3]. Recently a cytochalasin which inhibited the HIV-1 protease was discovered, indicating their potential as therapeutic drugs[4]. Study of the conformations

of these cytochalasins will undoubtedly foster the understanding of their biological activity.

2. THEORETICAL BACKGROUND

The expression "molecular mechanics" is currently used to define a computational method designed to give optimum structures and energies of molecules[5]. Owing to the importance of organic molecules, molecular mechanics has been extensively used to treat diverse classes of compounds. Unlike quantum mechanical approaches, electrons are not explicitly included in these calculations. This is possible due to the Born-Oppenheimer approximation, which states that the electronic and the nuclear motions can be uncoupled from one another and considered separately. Quantum mechanical methods have to do with the electronic system, after the nuclear positions have been established. The force field method involves the other part of the Born-Oppenheimer approximation, it assumes that the electrons in a system find their optimum distribution, and approaches chemical problems from the standpoint of the nuclear structure. A molecule from this perspective is considered to be a collection of masses that are interacting with each other via (almost) harmonic forces, and it is rather analogous to a system composed of weights joined together by springs. Potential energy functions are used to describe these interactions between nuclei. With judicious parameterization, the electronic system is implicitly taken into account. The method is a natural outgrowth from older ideas of bonds between atoms in molecules and of van der Waals and electrostatic forces between non bonded atoms. The basic idea is that bonds have "natural" lengths and angles, and

molecules will take up geometries having these values in simple cases. In addition, steric interactions are included using van der Waals potential functions. In more strained systems, the molecule will deform in predictable ways with "strain" energies that can be accurately determined.

2.1. Formulation of Molecular Mechanics

Early molecular mechanics calculations on the preferred conformations of ring molecules were performed using fixed geometries. The procedure was later refined by Wiberg[6] with the introduction of an energy minimization scheme whereby the energy minimum with respect to all the internal coordinates was sought.

Much of the early calculations, however, focused on the optimization of structures rather than calculating energies, or on applications directed towards a specific problem or class of molecules. Later on, two force field models appeared which are applicable to a wide variety of structural types and which have been parameterized against a large body of experimental data. Allinger and coworkers[7] have successfully improved their calculations for treating alkanes and have extended them to olefins, ketones, and conjugated systems, as well as applying them to the evaluation of a variety of problems in conformational analysis. A wide variety of thermodynamic and structural data have been reproduced with an impressive degree of accuracy. The other force field model is that of Boyd's group which was used to treat acyclic, cyclic, and polycyclic

alkanes, as well as some aromatic systems[8]. The Boyd force field, in contrast to Allinger's, has been developed to evaluate not only thermodynamic and structural data but vibrational frequencies as well.

Through successive stages of reparameterization and extension to an ever widening class of structurally diverse systems, a force field was developed by Allinger which is simple, generally applicable, extensively substantiated by experimental data, and which will accurately calculate the heat of formation structure of large organic molecules[9]. Complete description of molecular mechanics calculations, its applications and the usefulness, and limits of such calculations have been comprehensively reviewed by Burkert and Allinger[10].

2.2. Method of Calculation

Spectroscopic and chemical evidences indicate that the potential energy of large- or medium-sized molecules is approximately composed of energies localized in various parts of the molecule[11]. Thus, it has been customary to consider separately the harmonic forces of bond stretching, bond angle bending, the energy of twisting of torsion angles, and the energies of interactions between non bonded atoms (non bonded interactions).

2.2.1. Bond Stretching

Bond lengths are dynamic. The distance between the nuclei fluctuates with each vibration. There is, however, an optimal bond length around which the bond vibrates. The potential at any given interatomic distance l is described by the well known Morse curve, as shown in Fig. 1.1.

The Morse curve represents the interatomic attractive and repulsive forces. As two atoms are brought together from infinity, the protons of one attract the electrons of the other and their potential energy decreases until the nuclei are close enough to cause repulsive interactions overriding attractive forces of the atoms.

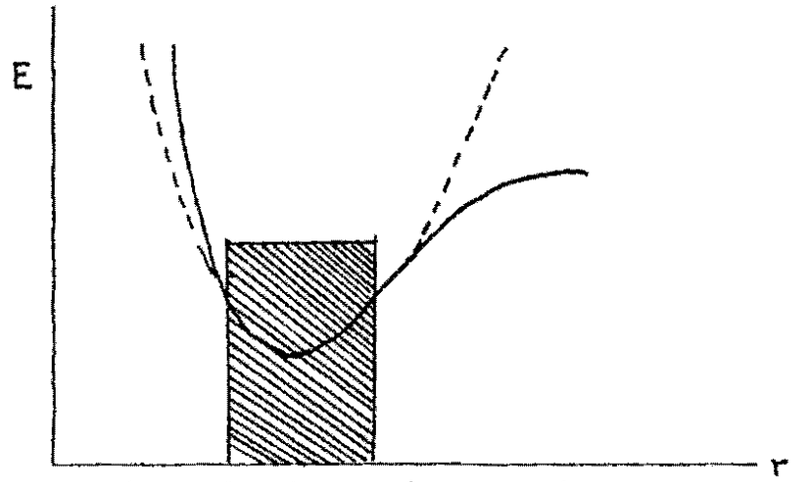


Figure 1.1. Harmonic and anharmonic functions: _____, Morse type of function, _ _ _, harmonic potential.

The vast majority of molecules have bond lengths within a limited range, symbolized by the shaded portion of Fig. 1. With in this part of the curve one of the simplest types of potential function, Hooke's law, gives a good fit to the energy profile, as shown by the dashed curve. The Hooke's law expression

$$E_n = \Sigma\ 1/2K(l-l_o)^2 \qquad 2.1$$

where E_s is the stretching energy and k is a constant, l_o is the natural bond length and the summations are over all of the bond lengths(l), is particularly simple to calculate and gives very fast execution of the program, so it is used in many force fields. The only problems that may arise are for molecules which may have bond lengths that lie outside the shaded area, in such cases the simple Hooke's law expression is no longer appropriate. A cubic term has been added to better reproduce the Morse curve in the region where bonds are being pulled apart[5].

$$E_s = \Sigma\ 143.88\ K_s/2(l-l_o)^2[1-2.00(l-l_o)] \qquad 2.2$$

The cubic constant has the value -2.00 times the quadratic constant. The factor of 143.88 converts the units to kcal/mol.

2.2.2. Angle Bending

Similar Hooke's law type potential functions summed over all of the bond angles (θ), are used to describe bond angle bending for small distortions. A cubic term has been introduced which has a substantial effect when the bending is more than about 10°-15°

$$E_b = \Sigma\ 0.043828\ K_b/2(\theta-\theta_o)^2[1-0.014(\theta-\theta_o)] \qquad 2.3$$

The Fourier coefficients have important physical interpretations. The one-fold term has been attributed to residual dipole-dipole interactions, the two-fold term arises from hyperconjugation in alkanes (or conjugation in unsaturated systems) and the three-fold term is steric[10]. Low periodicity (V1 and V2) torsional terms tend to be numerically much larger in systems carying heteroatoms than in hydrocarbons.

2.2.4. van der Waals Interactions

In principle a variety of equations can be used to describe van der Waals interactions. The Lennard-Jones 6-12 potential, Equation 2.6, has been used in many molecular calculations[6,7]. However in general the repulsive part of the 6-12 curve is too steep or hard to describe interaction between atoms in organic molecules over a wide range of distances[5].

$$E_{vdw} = \epsilon[(r_o/r)^{12} - 2(r_o/r)^6 \qquad 2.6$$

A better theoretical description of the repulsion expected between electron clouds is obtained by replacing the twelfth power term with an exponential[5].

$$E_{vdw} = \epsilon\{-2.25(r_v/r)^6 + 2.90(10^{-5} \exp[-12.5(r/r_v)]\} \qquad 2.7$$

The parameters needed are ϵ, the energy parameter that determines the depth of the potential well and r_v which is the sum of the van der Waals radii of interacting atoms.

2.2.5. Electrostatic Interactions

The electrostatic energy is calculated through either of the two usual formulas, i.e., to calculate the electrostatic energies from dipole-dipole interactions, according to Equation 2.8a.

$$E_{elec} = \mu_i u_j / Dr_{ij}^{\ 3}(\cos x - 3\cos\alpha_i \cos\alpha_j) \qquad 2.8a$$

where μ_i and μ_j are the dipole moments of the two bonds, x is the angle between them, r is the distance between their midpoints, and α_i and α_j are the angles between the bonds and the line joining their mid points, or from the charges on each atom as in Equation 2.8b.

$$E_{elec} = q_i q_j / Dr_{ij} \qquad 2.8b$$

The total energy, the steric energy, of a molecule is then the sum of the above contributing terms, i.e.,

$$E_{tot} = E_s + E_b + E_{tor} + E_{vdw} + E_{elec} \qquad 2.9$$

The steric energy can, therefore, be defined as the energy relative to a hypothetical molecule with the same constitution, but with all bond lengths, bond angles and torsion angles at their "strainless" values, and the atoms with van der Waals and electrostatic interaction corresponding to infinite separation of the atoms[10].

2.2.6. Cross Terms

It is known that when a bond angle is reduced, the two bonds forming the angle will stretch in order to alleviate the resulting strain. A stretch-bend term is added to account for this effect.

$$E_{sb} = 1/2K_{sb}[(l-l_o) + (l'-l_o)](\theta-\theta_o) \qquad 2.10$$

Here l and l′ are the two bonds that are joined to a common atom, and θ is the angle between the two bonds. To better fit vibrational frequencies, torsion-stretch, bend-bend and torsion-bend terms have been incorporated into more refined programs[5].

2.3. Force Fields for Delocalized Pi Electrons

Conjugated systems pose a special problem not discussed in earlier sections. The bond length in these systems vary with the number of double bonds and are determined by the bond orders. To correctly reproduce the structure of conjugated molecules in general, the effects of bond order variation in different systems must be considered. This requires a self-consistent field (SCF) calculation on the pi system to obtain the bond orders which are used to assign stretching and torsional parameters of the unsaturated system[12]. The natural bond length l_o, required by the force field is obtained from the linear relationship:

$$l_o = 1.512 - 0.179\ p_{ij} \qquad 2.11a$$

Where p_{ij} is the calculated SCF bond order. The stretching force constants, k_s, for conjugated bonds are calculated by

$$k_s = 5.0 + 4.6p_{ij} \qquad 2.11b$$

The two-fold torsional constants are related to pij and β_{ij}° as shown in Equation 2.11c

$$V_{2ij} = 16.25 \times f \times p_{ij}\beta_{ij}^{\circ} \qquad 2.11c$$

where $V2_{ij}$ is the two-fold torsional constant across bond i-j and $p_{ij}\beta_{ij}^{\circ}$ is the product of bond order and resonance integral (β) across bond i-j in the corresponding π-planar conformation and f is given by

$$f = \Sigma_{j<j} (p_{ij}\ \beta_{ij}^{\circ} - p_{ij}\beta_{ij}(\text{real}) / \Sigma_{i<j} p_{ij}\beta_{ij}^{\circ}(1-\cos\omega_{ij}) \qquad 2.11d$$

for non planar unsaturated structures where the sum is over all π-bonds, $p_{ij}\beta_{ij}$(real) is the pβ product across bond i-j in the real (distorted) Π system and ω_{ij} is the dihedral angle across i-j. f is unity for planar structures. The steric energy is then minimized with respect to geometry using Newton-Raphson scheme discussed below. If the geometry has been significantly changed during the minimization step, the SCF calculations are repeated and the new bond orders are used to assign new stretching and torsional parameters.

3. GEOMETRY OPTIMIZATION

The energy of a molecule of any trial geometry can be calculated utilizing equations of the form given above. The optimum structure of the molecule will correspond to that geometry where the energy is at a minimum. Therefore if the total energy of the molecule is given as in Equation 2.9 the structure is found by taking the derivative of this equation with respect to each of the degrees of freedom of the molecule, and find the place (or places) at which each of those derivatives is simultaneously equal to zero.

Energy minimization methods are used to refine molecular structures in the sense of reducing worst steric conflicts and adjusting bond lengths and bond angles to values near their respective optima, but they generally will not produce structures that are very different from the initial, unrefined structure.

Given a set of independent variables $X= (x_1,x_2...x_n)$ and a specified objective function $V = V(x)$, the task is to find the set of values for the independent variables X^*, for which the function has its minimum value $V(X^*) = \min(V(X))$. In the case of a model molecule containing N atoms, the 3N component of X are the atomic coordinates and V is the potential energy, calculated from the energy function. The two commonly used minimization techniques are steepest descent and Newton Raphson methods.

Steepest descent method consists of moving over the energy hypersurface in the direction defined by the gradient of

largest magnitude until the minimum is found. Wiberg, in the first general computer program for energy minimization, used the steepest descent method with numerically calculated first derivative[6]. It is an iterative process, where each iteration consists of three parts. First, a descent direction is chosen; this can be represented by 3N dimensional vector of unit length S_k. Second a descent step size, specified by scalar λ_k, is determined. Third, the descent step is taken according to the relationship

$$X_k = X_{k-1} + \lambda_k S_k \qquad 3.1$$

The conformation of minimum energy can be obtained by computing the energy, and energy gradient from the initial trial coordinates. Then these independent coordinates are changed by a small amount. From these new coordinates the energy and its gradient are computed again. The same cycle of computation is repeated until the minimum is reached.

The Newton Raphson method is a second derivative technique in which the cartesian coordinates of the atoms are adjusted to converge upon the minimum energy position. Let x^m_i (i=1,3) represent the cartesian coordinates of a particular atom at the minimum energy position and x^t_i the trial position somewhat in the vicinity of the minimum. The partial derivatives of the energy, E, with respect to x_i may be expressed as the truncated Taylor series

$$\partial \frac{E}{\partial x_i^m} = \partial \frac{E}{\partial X_i^t} + \sum_{J=1}^{3} \partial^2 \frac{E}{\partial X_i^t \partial X_j^t} \cdot \delta X_j \qquad 3.2$$

where $\delta x_j = x_j^m - x_j^t$.

The minimum energy conformation corresponds to a solution of the equations $\nabla E\ (x^i) = 0$ so that

$$\partial \frac{E}{\partial X_i^t} = \sum_{J=1}^{3} \partial^2 \frac{E}{\partial X_i^t \partial X_I^t} \cdot \delta X_j \qquad 3.3$$

and the correction to the trial position is given by

$$\delta x_j = -D^{-1}{}_{xixj} D_{xj} \qquad 3.4$$

where D is the 3 X 3 matrix of second derivatives and **D** the vector whose components are the first derivatives. These equations can be solved in δ_{xj} for each of the atoms in turn, after introducing the appropriate constraints to define the position and orientation of the molecule, and the calculation iterated until the corrections become sufficiently small. The calculation of the derivatives was carried out numerically in some early programs[13], but this is done analytically in more recent programs[14,15].

At the energy minimum the above equation is six fold singular, due to the three translations and three rotations which leave the potential enrgy of a free molecule unchanged.

Eckart conditions, which fix the center of mass and constrain for infinitesimal ratations, are imposed on ΔX_i:

$$\Sigma^{N}_{i=1} m_i \Delta X_i = 0$$

and

$$\Sigma^{N}_{i=1} m_i (x_i \Delta Y_i - y_i \Delta X_i) = \Sigma^{N}_{i=1} m_i (y_i \Delta Z_i - z_i \Delta Y_i)$$

$$= \Sigma^{N}_{i=1} m_i (zi \Delta X_i - x_i \Delta Z_i) = 0$$

where m_i is the atomic weight of the ith atom; x_i,y_i and z_i are the tree components of the ith position vector and ΔX_i, ΔY_i and ΔZ_i are the cartesian displacement coordinates. These additional equations may be combined with the above equations in to a single set of equations by using the method of lagrange multipliers.

Interconversion between internal coordinates and cartesian coordinates can be effected through the following equations. The bond length and the non bonded distance between atoms i and j is given by

$$r_{ij} = [(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)]^{1/2} \qquad 3.5a$$

the angle between atoms ijk

$$\cos\theta_{ijk} = - \mathbf{r}_{jk} \cdot \mathbf{r}_{ij} / r_{jk} r_{ij} \qquad 3.5B$$

and the dihedral angle between atoms ijkl

$$\cos\omega_{ijkl} = (\mathbf{r}_{jk} \times \mathbf{r}_{ij}) \cdot (\mathbf{r}_{kl} \times \mathbf{r}_{jk}) / r_{ij} r_{jk}^2 r_{kl} \sin\theta_{ijk} \sin\theta_{jkl} \qquad 3.5c$$

4. CONFORMATIONAL ANALYSIS

As described above optimization techniques in molecular mechanics will afford only a single energy-minimized structure. However, that structure may not correspond to the global minimum and there may be multiple minima on the conformational space: the multiple minimum problem. This problem stems from the intrinsic difficulty of locating the populated, low-energy conformers. To perform a "conformational search" it is therefore necessary to determine those minimum-energy conformations that are believed to contribute to the overall conformational partition function[5]. This requires some means of determining the energy of any given conformation and a method of deriving initial structures for subsequent minimization.

A variety of methods have been proposed and used for exploring the conformational space available to molecular systems. These methods differ primarily in the scheme used to generate the starting geometries. The methods may be classified by the coordinate system used to describe the molecular geometry and by the method used to vary the coordinates.

Torsion angles (internal coordinates), cartesian coordinates (external coordinates), and matrices of internuclear distances (distance geometry) are the coordinate system which have been used most frequently. Apart from these methods molecular dynamics begins with an initial low-energy structure and integrates Newton's second law of motion ($F = ma$) at regular time intervals. New positions and velocities of

atoms are calculated, the atoms are then moved to these new positions and the cycle repeated. The time periods over which many conformational changes occur, however, are rather long and as a result molecular dynamics is most useful as a method for local conformational searching[5].

4.1. Conformational Searching Using Internal Coordinates

It is obvious that conformational stereoisomers differ primarily in their torsion angles. The bond lengths and angles of different conformers vary relatively little, because they are defined by stiff, single-minimum potentials[16]. The use of internal coordinates thus facilitates selective sampling of the low-energy regions of conformational space where stable conformers are found.

The two common algorithms for searching conformational space employing internal coordinates are the systematic or grid searching and random search (Monte Carlo) methods.

4.1.1. Systematic Search Method

The conformations of a molecule can to a first approximation be defined as those structures that differ solely by rotation about single bonds. An obvious way to perform a conformational search is to systematically increment each single bond through 360°, thereby generating all possible combinations of torsion angles. Such an algorithm is called a grid search. This method performs the angular search by

choosing the rotatable bonds in a sequential unidirectional fashion. In an acyclic molecule the search starts at one end of the structure and alters succeeding dihedral angles as one moves down the chain. In a cyclic system, rings are opened, for purpose of conformer generation, to give a 'pseudoacyclic' molecule, which is then processed as for the acyclic case, with ring closure constraints enforced where appropriate[17,18]. In MULTIC[17], for example, six values must be within prescribed limits for the conformation to be acceptable. The most important of these, the closure distance constraint, is simply a range of acceptable distance between the two atoms forming the ring closure bond. Two bond angles and three dihedral angles comprise the remaining closure dependent internal coordinates and are constrained for final structure selection as shown in Fig. 4.1.

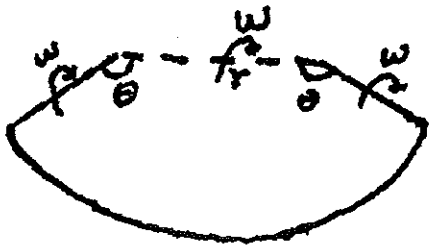


Figure 4.1. Closure parameters: one bond length, two internal angles, and three torsion angles.

4.1.2. An Internal Coordinate Monte Carlo Method

An alternative to the full-space, systematic search is a search based on random variation of the internal coordinates. A randomly selected torsion angle is rotated by a randomly selected increment and the resulting structure is optimized by energy minimization. This method depends on the idea that

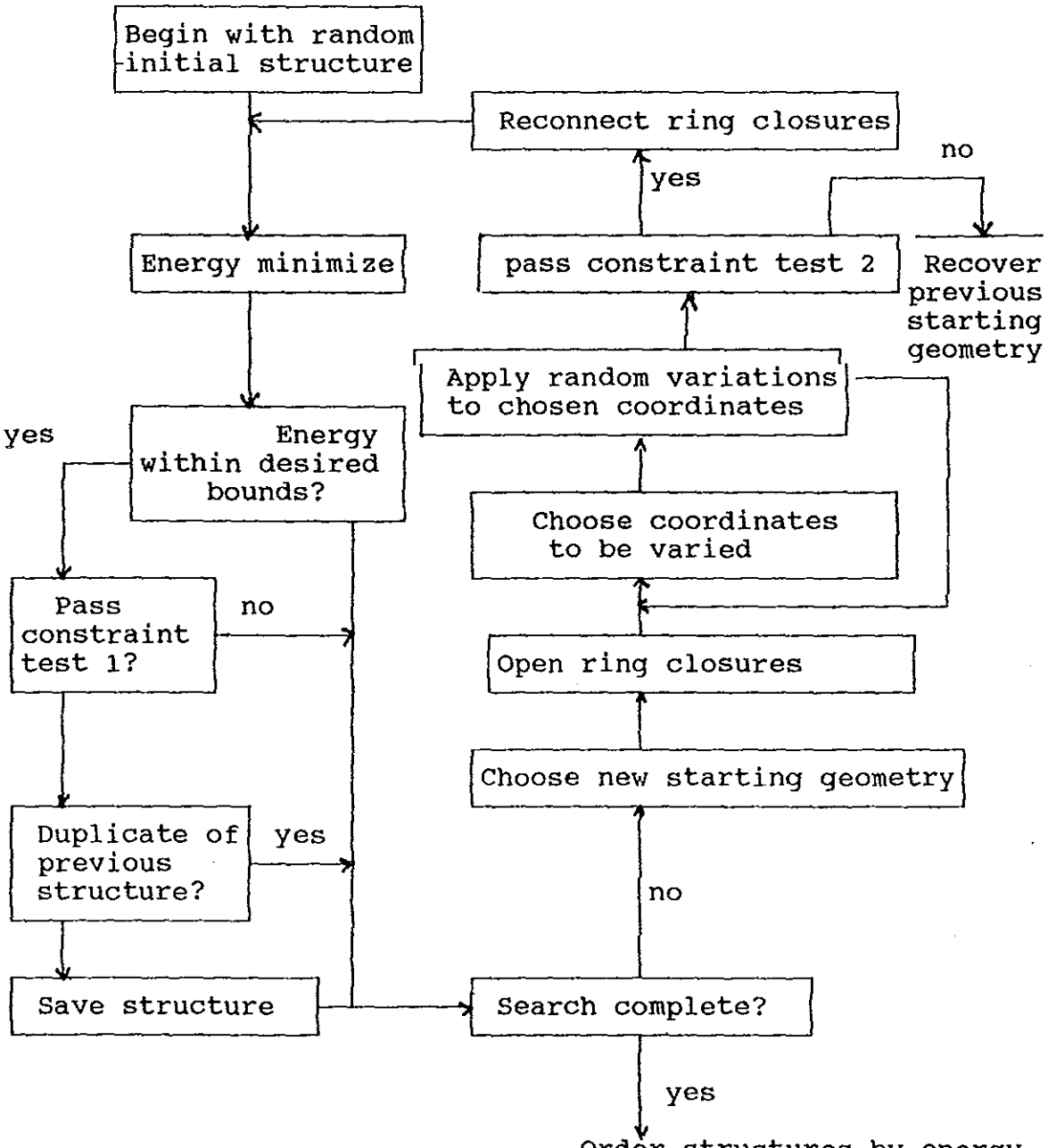
energy minimization finds local energy minima and that random sampling of the potential energy surface will more rapidly search the surface.

For a molecule with n rotatable bonds and for an angular increment of θ, the number of conformations generated is therefore $(360/\theta)^n$. This number quickly becomes very large and requires very long computing time. Because of this, some representative rotational angles are selectively used[19]. For an amide bond, the resolution set have two angles (0° and 180°). For an sp^3-sp^3 bond, three angles are used (0°, 120°, and 240°). For an sp^3-sp^3, twelve angles are considered (0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330°). For an sp^2-sp^2 six angles are chosen (0, 60, 120, 180, 240,and 300°).

In conducting a conformational search a starting structure is chosen, random variation of these selected coordinates are applied, the structure is minimized and the result compared with minima found during previous conformational search steps. After the resulting structure has been stored as a new, unique conformer or rejected as a duplicate the cycle is repeated. The algorithm used for the Monte Carlo minimum search is summarized in the flow diagram shown in Scheme 4.1.

Constraint test 1 is used to eliminate energy-minimized structures (1) whose energies lie outside the selected energetic upper bound relative to the instant global minimum, (2), which have inverted chiral centres. Constraint test 2 eliminates starting geometries having poor ring-closure characteristics or high energy non bonded contacts. Structures are rejected primarily by interatomic constraint

test which eliminate chemically unreasonable geometries. Since the severity of steric interactions between atoms separated by three bonds is effectively limited by realistic values of intervening bond distances and angles, 1,3- and 1,4-non bonded interactions are not subjected to non bonded constraint testing[17].



Scheme 4.1. Flow diagram of the conformational searching algorithm.

A 1,5-interaction represents the most closely connected non bonded arrangements. A 3Å cutoff is applied between non hydrogen atoms. Structures are defined as duplicates if no fit better than 0.1Å RMS is obtained from least-squares atom by atom superposition of each minimum-energy conformer[18].

4.2. Cartesian Stochastic Search

The stochastic or Monte Carlo search method on cartesian coordinates employs a random translation algorithm to generate starting geometries. It operates by applying limited, random translations or "kicks" to the cartesian coordinates each atom of known conformations to give new starting geometries. The resulting structure is minimized, and then the "randomization" process is performed again. Implementations of this searching scheme have been described by Saunders[20] and Ferguson and Raber[21]. In this method, the x, y, z, coordinates of each atom in the molecule are perturbed individually by addition of a random increment uniformly distributed between $\pm\delta$, where δ a specified value[22,23]. Saunders used larger increments (up to 3.5Å) than Ferguson and Raber, which suggested a maximum of 1.7Å.

6. EXPERIMENTAL

The purported structure of the novel cytochalasin from fungus Xylaria obovata is shown below in Fig. 6.1. The molecule was analyzed as two fragments--the benzyl moiety and the cytochalasan base.

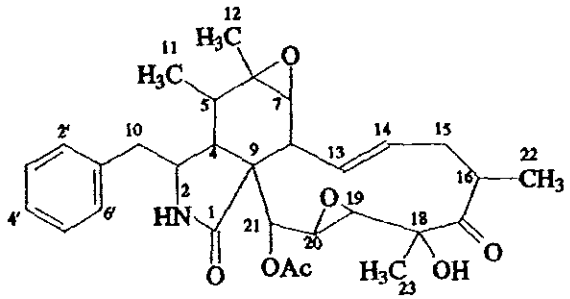


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Fig. 6.1. Structure of the new fungal cytochalasin

The cytochalasan base was input from two fragments, the isoindole ring and the 11-membered macrocycle, which were fused with the stereochemistry reported[1]. These fragments were drawn using the input routine of PCMODEL[29]. The 5- and 6-membered rings were fused in a cis orientation and the epoxide at C19-C20 was trans. The six-membered ring was input as a geometry optimized to a twist-boat conformation.

The 11-membered macrocycle was initially input using a trial structure maintaining the steric bulk of the benzyl and acetyl substituents by using methyl substituents to decrease the computational time. This fragment was energy minimized and was used as starting structure to search for other low-energy conformations using GMMX[19]. The lowest energy conformation

found in this search was fused in a trans orientation to the isoindole ring, and was used as the input for the cytochalasan core.

7. RESULTS AND DISCUSSION

The novel fungal cytochalasin studied here contains 37 heavy atoms, three fused rings, an aromatic ring and several substituents. Initial conformational analysis on this big molecule did not give converged results because of the large conformational space accessible to the molecule. The structures thus found included many high energy forms which do not contribute significantly to the properties of the compound. Particularly, the high mobility of the acetate group precluded the exploration of various conformations of the other part of the molecule.

Due to the size of the molecule and the presence of the aromatic ring (which requires VESCF calculations for determining the optimum values of the parameters in the force field equation as described above), the time required to complete a single run was considerably long, over three days of CPU time on a 486 class computer. Since several such runs were necessary to complete the search for low-energy regions of conformational space, the search was carried out more efficiently in steps as described below.

The conformational analysis began with finding the low-energy orientations of the benzene ring relative to the five-membered lactam ring. The eleven- and six-membered rings are too distant to have any steric effects of consequence, and they were deleted to save computing time. Free rotation about the two adjacent rotatable bonds of the benzylic carbon gives rise to infinite orientations. Grid searching about the rotatable

bonds has been carried out on the fragment below, Fig. 7.1., and three low-energy conformations were obtained.

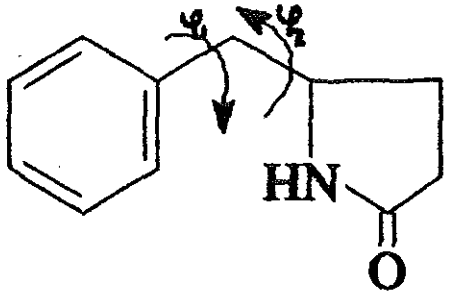


Fig. 7.1. The fragment studied for locating the orientation of the benzene ring relative to the cytochalasan core.

Because the benzyl fragment is a flat molecule with C_{2v} symmetry, the rotation was limited to 180°, the other half being the mirror image of the first. This ring was rotated about φ_1 in 30° increments over 180° rotation with the energy calculated at each grid point. For each grid point of the benzene ring the lactam ring was subjected to a full 360° rotation about φ_2 and the energy calculated at 30° increments. The smoothed contour diagram of the energy versus the two dihedral angles φ_1 and φ_2 shows three minima, as shown in Fig. 7.2. After determining the low-energy conformations of this part of the molecule, the next conformational analysis was limited to the cytochalasan core. To speed the conformational searching and limit the generated conformations to a manageable number, the search was directed toward the more populated, low-energy regions of conformational space by restricting the

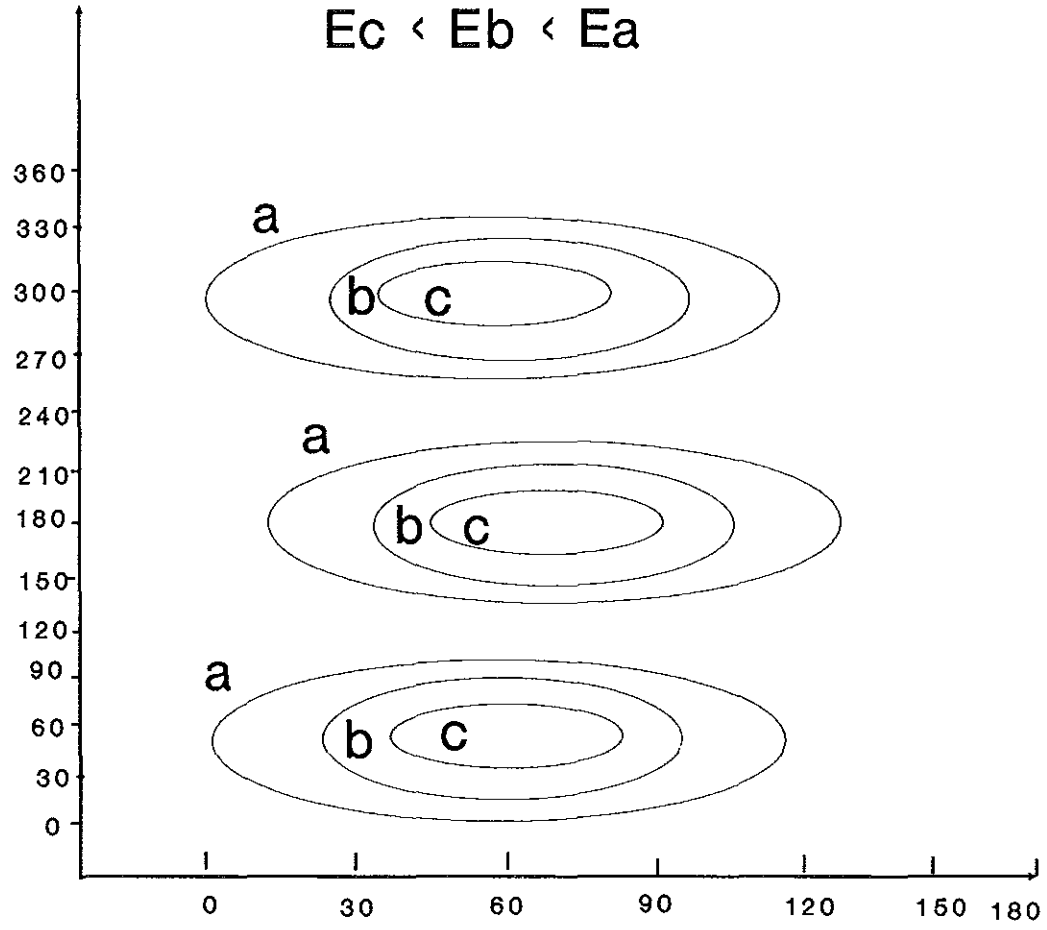


Fig.7.3 Contour diagram of energy Vs. the two angles

retained conformations to 10 kcal/mol or less above the minimum. This procedure favoured the formation of new conformations which are low in energy. The isoindole moiety and the 11-membered ring were studied separately using this approach. The lowest energy conformations found were fused maintaining the trans orientation and used as starting structure for further conformational study of the cytochalasan core.

The search was then aimed at identifying the cytochalasin global minimum and those possible conformations within 10 kcal/mol of it. This energy gap is too large to describe only those minimum energy conformations that are believed to contribute to the overall population at room temperature. However, it gave possible solutions to the problem of the preferred conformation of the six-membered ring (see below), and furthermore it was informative in predicting possible intramolecular hydrogen bonding in cases where there are large conformational differences.

A total of 22 conformations were generated within this energy gap of 10 kcal/mol. Although these conformations were minimized to different local minima, and hence have different energies, there are certain features of the conformations upon which one could group similar conformations together. These features are:

1) The conformations of the six-membered ring,

2) The relative orientations of the plane containing the double bond and the plane though the carbons bearing the epoxide group on the 11-membered ring,

3) The dihedral angle between H7 and H8 which is additional information in differentiating the conformations of the six-membered ring,

4) The presence or absence of intramolecular hydrogen bonding between the hydrogen of the hydroxyl group at C18 and the oxygen of the epoxide or the ketone,

5) The angle between the line through the ketone group and the line through the carbons of the epoxide.

On the basis of these factors, cluster analysis[30] has been done for the preliminary analysis and groupings of the 22 conformations. The Square Euclidian distance based on complete linkage method was used as a measure of similarity for clustering of similar conformations at different stages of the analysis. Analysis of the members of the clusters indicated that some conformations have similar three-dimensional structures, with minor differences which could be attributed to slight variations in bond angles and torsion angles. They do, however, differ enough in conformation that their geometries were not optimized to the same minimum energy.

Five clusters were obtained at the stage where the value of the coefficient (the distance at which two clusters are combined) showed the first large change. These five clusters were grossly analyzed, from which the following general description of the various conformations was drawn.

7.1. Description of Conformer Geometries

The resulting geometries of the 22 cytochalasin conformers are described below and tabulated in Appendix 2. The cytochalasin geometry attributes are grouped below by ring fragment.

7.1.1. The Six-membered Ring

The boat conformation is the most preferred conformation of this ring as the nineteen lowest-energy conformers are in the boat form. Detailed information is given below. It is only the three highest energy conformers which vary by adopting a half chair form. It can, therefore be concluded that the boat form is the preferred conformation. The measured torsion angle values for boat and half chair conformations are given in Appendix 3.

7.1.2. The 11-membered Ring

It is in this part of the molecule that most of the conformations differ from each other. This is due to the possibility of free rotation about the various single bonds. As a result the line through the two carbons of the oxirane ring (C19-C20) and the line of the olefinic double bond vary in relative orientation from nearly parallel to perpendicular orientations in the different conformers obtained.

There also exists the possibility of intramolecular hydrogen bonding between the hydrogen of the hydroxyl group at C18 and the oxygen of the epoxide at C19-C20. Five out of the 22 conformations have such a hydrogen bonding. The other potential hydrogen bonding acceptor is the oxygen of the ketone group at C17, where it was detected in three conformations. Most of these conformations have high energy indicating unfavourable structural changes are required to bring the donor and the acceptor close to each other.

7.2. Comparison of Cytochalasin Structures

The structure of the global minimum conformation of the novel fungal cytochalasin was compared to other similar cytochalasins. The cytochalasins described as B, E, G, D', and H vary in macrocyclic ring size, aromatic ring substituent, and functionality (see Fig. 7.3)

7.2.1. The Isoindole Moiety

There are four possible conformations of the six-membered ring, i.e., chair, half-chair, boat, twist-boat. Together with the number of chiral centers found in this ring, several possible isomers can be generated. The NMR data has been used as the first approximation to the solution of the problem[32]. The stereochemistry of cytochalasin B was solved by quantitative application of the relation determined by Karplus[24] between the dihedral angle and the NMR coupling

constant for vicinal protons. It was possible to rule out some conformations and hence to reduce the possible conformations of the fragment to four. However, it was not possible to distinguish these four conformations on the basis of NMR data alone, because the values of coupling constant for the coupled protons in these conformations would be similar. It was only on the basis of x-ray analysis[31,32] that the preferred conformation was known to be boat. Unlike the cytochalasin under consideration, in these cytochalasins there is an exocyclic double bond between C6 and C12. This sp^2 hybridization at C6 has been given as a reason for preference of the boat conformation[32]. The same conformation is reported for cytochalasin G having an epoxide group at C6-C7 position[33].

For the novel fungal cytochalasin the conformation of the six-membered ring in the lowest-energy conformer as well as in most of the conformers obtained is clearly in the boat form. Since coupling constant is directly related to the geometry through dihedral angle, the calculated vicinal coupling constants for the protons on the six-membered ring were compared with the experimental values of cytochalasins K, L, M, with epoxide group at C6-C7[34](Table 7.1).

The calculated coupling constants are the averages of the six lowest-energy conformations whose Boltzmann populations are above 1% (see Appendix 4).

Table 7.1. Comparison of Coupling Constants

	cyt K	cyt L	cyt M	Cal. for I
J_{3-4}	2.5	3.3	4.0	3.66
J_{4-5}	5.5	4.5	4.0	3.60
J_{7-8}	5.0	-	5.5	5.85

The calculated values are within acceptable range to the experimental results suggesting similarity in the conformation of this ring. Complete information regarding the three-dimensional structure of the heavy atoms in the fused five- and six-membered rings has been obtained from the x-ray data of some cytochalasins[32]. As the conformation of a molecule is determined by the orientations of the bonds in space, defining all the torsion angles involved can describe its geometry. The measured torsion of the isoindole moiety have been compared with the experimentally determined values of cytochalasins B, E, G, D′ and H (Table 2). The structures of these compounds are given in fig. 7.3.

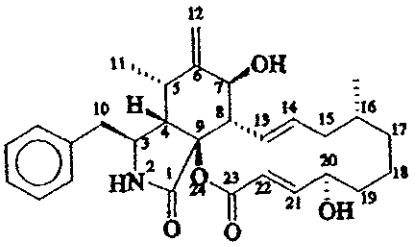
Table 7.2. Comparison of Isoindole Dihedral Angles

dihedral angle	defining atoms	B	E	G	D′	H	global minimum
a	9-1-2-3	3.4	-19.3	-5.6	4.3	-1.3	-0.16
b	1-2-3-4	-2.5	21.7	13.9	4.8	6.8	4.01
c	2-3-4-5	115.1	109.1	106.0	111.7	114.2	120.83
d	3-4-5-6	-68.7	-66.8	-70.9	-70.0	-69.2	-70.00
e	4-5-6-7	-58.8	-57.3	-54.7	-63.4	-59.9	-48.08
f	5-6-7-8	11.7	3.1	2.7	16.2	10.2	-4.37
g	3-4-9-8	125.1	129.9	106.0	111.7	114.2	122.54
h	6-7-8-9	44.1	50.6	52.7	42.6	46.7	53.93
i	7-8-9-1	65.2	69.0	58.3	59.8	61.5	63.48
j	2-1-9-4	-2.8	9.2	-5.4	-11.6	-4.7	-3.67

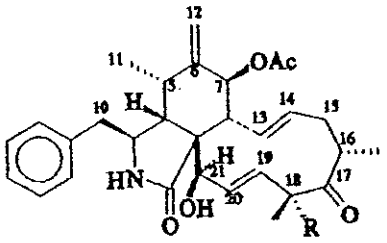
It can be seen that the calculated values are in good agreement with the experimental values, therefore the global minimum energy conformer geometry resembles the indole geometry of the

other cytochalasins. The fusion of the five- and six-membered rings was initially constrained to be cis, the geometry reported for all the cytochalasins mentioned above. The trans fusion seems highly strained and has not been considered[35]. The five-membered ring is significantly puckered toward the six-membered ring. C5 and C8 are constrained to be eclipsed (dihedral angle -4.11) by the fusion to the lactam ring at C4 and C9. With regard to the stereochemistry of the six-membered ring, Vurro et al.[36] correlated the coupling constants between H-7 and H-8 and between H-4 and H-5 to those recorded for the same couplings in cytochalasins having a ß-epoxy group located between C-6 and C-7 to assign the stereochemistry of cytochalasin F. On the basis of this correlation a similar comparison of the calculated coupling constant values between H-7 and H-8 (J=5.85) and H-4 and H-5(J=3.6) shows these values are not far from the couplings in a cytochalasin having a ß-epoxy group located between C-6 and C-7[34,36] and hence, a ß-configuration may be deduced for the oxirane ring, H-4, H-5 and H-8, consequently, an α-configuration for H-7, Me-11 and Me-12. This fact was also reported by Dagne et al.[1].

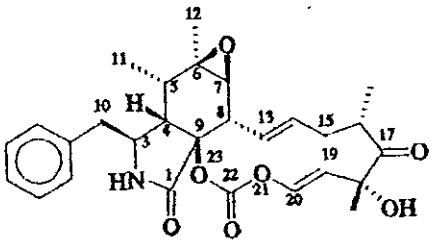
After examining 5,6-epoxidic steroids, Cross[37] has suggested that the angles (φ) subtended by the epoxidic C-H bonds with the bonds at C7-H are sufficiently different to permit a differentiation between the α- and ß-epoxides to be made through a study of J values.



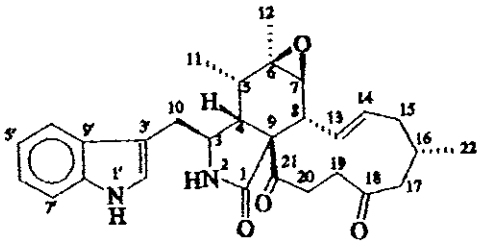
B



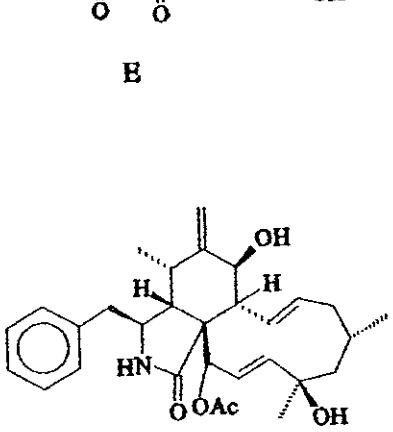
D'



E



G



H

Fig. 7.3. Structures cytochalsins B, E, G, D' and H

This simple but highly informative correlation could not be extended to the structure under consideration because of the difference in the conformations of the six-membered ring in these two cases. The six-membered ring adopts a chair conformation in steroids and a boat structure in the cytochalasins.

7.2.2. The Macrocycle

In the eleven-membered ring of the global minimum the C13-C14 trans double bond and the C19-C20 bearing the epoxide group are almost parallel to each other, similar to that reported for cytochalasin H, which has two double bonds at these positions . The calculated J_{8-13} = 11.28 is close to the experimental value (J=10) and to other cytochalasins[34,36]. This shows that the constrained fusion to the six-membered ring at C8 and C9 was likely. From the J values of H8-H13(J=10) and H8-H14(J=0) it has been deduced that the C13-C14 double bond is at right angle to the principal plane of the six-membered ring[38]. The calculated value for H8-H13 is (11.28) suggesting similar orientations. The couplings from proton on C(20) to the proton on C(21) as found from experiment[2] is very small (0.8) from which a dihedral angle close to 90° was suggested. The same value is reported for this coupling for Engleromycin[39], a cytochalasin very similar to I except that it has a hydroxyl group at C21 instead of an acetyl group. Nagakawa et al.[40] have formulated a simple equation relating the coupling constant to the dihedral angle between the

epoxidic proton and the proton on adjacent carbon atom for $0^\circ \leq \theta \leq 90^\circ$.

$$J = 5.1 \cos^2\varphi \qquad 7.1$$

The measured dihedral angle(of the global minimum) is 64° which gives a coupling constant of 0.98. The average value for this coupling is 0.84, fairly close to the experimental value. The calculated and experimental values of the various coupling constants is summarized in Table 7.3.

Table 7.3. Experimental and calculated coupling constants

	3H4H	4H5H	7H8H	8H13H	14H15a,b	16H15Ha,b	20H21H	3H10Ha,b
Jexp	2.0	5.8	5.6	10.0	6,10.6	-	0.8	6,8
Jcal	3.7	3.6	5.9	11.3	4.4,10.2	4.3,11.3	0.84	2.1,3.7

The whole conformation of the 11-membered ring has been compared through the values of the various torsion angles reported for cytochalasins B, E, D′, and H (Table 7.4).

Table 7.4. Comparison of the dihedral angles of the macrocycle

	Defining atoms	B	E	G	D′	H	cal. for I
l	8-13-14-15	171.1	180.0	181.3	177.7	181.6	179.69
m	13-14-15-16	-129.1	-127.2	-124.8	-91.2	-103.1	-99.31
o	14-15-16-17	76.6	68.2	55.5	73.7	71.0	64.44
p	16-17-18-19	172.7	66.3	151.2	66.1	65.3	67.94
q	17-18-19-20	-101.4	134.7	-67.1	76.0	84.8	94.88
r	18-19-20-21			-75.0	-175.0	-173.1	-160.1

The torsion angle values of these cytochalasins fall within close range. Likewise the calculated values for I roughly parallel (with not too far deviation) the experimentally observed values of the other molecules.

The portion of the large ring, starting at C8 and counting to C18, has a fairly constant conformation in spite of the different sizes and expected flexibility of these rings, and as a result, it can be concluded that the 11-membered ring is in a preferred conformation. The values of the various bond lengths and bond angles for the lowest energy conformer are given in Appendix 5.

8. CONCLUSION

Performing a "conformational search" demands as the minimum input, information regarding the types of atoms and their trial coordinates that define the molecular structure. The energy of the trial structure generated was calculated by the force field equations and minimized to the local minimum. This structure was then submitted to the conformational searching algorithms to identify minima on the potential energy surface. However, because the number of minima increases dramatically with the number of rotatable bonds, it was impossible to explore all the minima on the surface described by the potential energy surface from a single trial geometry. It was then necessary to reduce the scope of the search and this was done in two ways.

The first was to impose some form of constraint on the conformations generated. These constraints came from experimental investigations. The fusion of the five-membered and the six-membered rings of the isoindole part of the molecule was constrained to be cis and the fusion of the macrocycle to the isoindole moiety was also constrained to be trans. The second way the search was reduced to a manageable level was to bias it toward regions of the conformational space that correspond to the very lowest energy structures.

There are some deviations in the value of the calculated coupling constants from the experimental values. This could be due to the inherent limitations in the equations developed to calculate the coupling constants and the absence of solvent effect, because the calculations are carried out for the

isolated gaseous molecule. Since most of the calculated values for the various parameters are in a fair agreement with the experimental values it can, therefore, be concluded that the likely three-dimensional structure of the new cytochalasin has been reproduced through theoretical calculations.

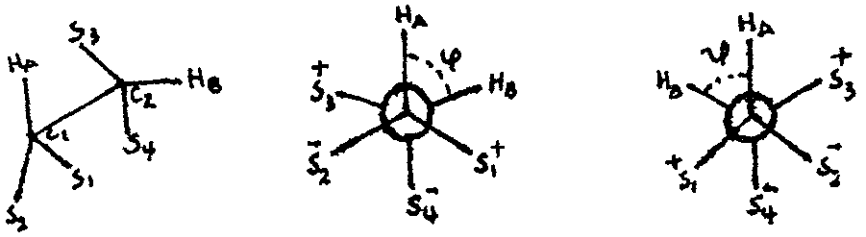
Appendix 1

I.The values of the empirical parameters P1-P7*

parameters	no substituents	including β-effect	no. of substituent		
			2	3	4
P1	13.86	13.70	13.89	13.22	13.2
P2	-0.81	-0.73	-0.98	-0.99	-0.91
P3	-	-	-	-	-
P4	0.56	0.56	1.02	0.87	0.53
P5	-2.32	-2.47	-3.40	-2.46	-2.41
P6	17.9°	16.9°	14.9°	19.9°	15.5°
P7	-	0.14	0.24	-	0.19

* reproduced from reference 24

II. Definition of "positive" and "negative" substituents.



Projecting the H_A-C1-C_2-H_B fragment along the vectors C_1C_2, the orientation of a substituent S on C_1 as being positive when the projected valence angle between H_A and S amounts to approximately +120°, counting clockwise from H_A. The orientation of a substituent is negative when the angle amounts to 240°. This definition determines the sign of each substituent independent of the instant value of θ.

Appendix 2

Summary of description of the 22 conformers from cluster analysis

cluster no.	struct. no.	steric E kcal/mol	conf. of six-mem. ring	H bond	orientation of C13-C14 and C 20-C21 bonds
1	20*	47.82	half chair	all	nearly parallel
	21	48.74			
	22	48.80			
2	8	42.25	boat	none	nearly perpendicular
	12	43.21			
	18	46.02			
3	1	38.89	boat	2, 10	no regularity
	2	39.45			
	4	40.19			
	10*	42.93			
	16	45.24			
4	7	42.03	boat	9,14,17	nearly perpendicular
	9	42.45			
	11	43.25			
	13	43.90			
	14	44.92			
	17*	45.38			
5	3	40.18	boat	none	nearly parallel
	5	40.21			
	6	60.34			
	15	45.05			
	19	47.35			

* Hydrogen bonding with the ketone

Appendix 3

Torsion Angles in Boat and Half Chair Conformations

Defining atoms	Boat	Half Chair
4-5-6-7	-48.32	44.48
5-6-7-8	-3.75	-7.98
6-7-8-9	53.36	-17.27
7-8-9-4	-47.83	4.77
8-9-4-5	-3.35	30.61
9-4-5-6	51.94	-56.62

Appendix 4

Coupling Constants Averaged Over Six Low-Energy Conformations

Str.no	1	2	3	4	5		
Boltz. distr.	54.40	20.85	6.44	6.28	5.67	5.17	J ave.
J_{3-4}	3.50	3.45	4.87	2.89	5.88	3.66	3.66
J_{3-10a}	2.19	2.20	2.10	2.20	2.02	2.12	2.09
J_{3-10b}	3.83	3.83	3.94	2.83	4.04	3.83	3.72
J_{4-5}	3.80	3.62	3.09	3.91	2.82	3.40	3.60
J_{7-8}	6.00	5.92	5.42	6.10	5.80	5.78	5.85
J_{8-13}	11.40	11.46	11.53	11.55	11.03	11.6	11.28
J_{14-15a}	5.00	4.48	2.62	4.92	2.78	2.68	4.42
J_{14-15b}	10.12	10.75	10.44	10.02	11.09	10.86	10.18
J_{16-15a}	4.83	2.17	5.76	1.84	5.93	5.59	4.20
J_{16-15b}	11.13	12.12	11.20	12.75	11.14	11.24	11.30
J_{20-21}	0.98	0.84	0.29	0.98	0.59	0.25	0.8

Appendix 5

I. Bond Angles in (deg.)

O(1)-C(1)-C(9)	127.43	C(1)-C(9)-C(4)	100.06
O(1)-C(1)-N(2)	119.06	C(1)-C(9)-C(8)	108.64
N(2)-C(1)-C(9)	113.51	C(1)-C(9)-C(21)	110.75
C(1)-C(2)-C(3)	113.88	C(4)-C(9)-C(8)	112.09
N(2)-C(3)-C(4)	103.83	C(4)-C(9)-C(21)	110.25
N(2)-C(3)-C(10)	111.65	C(8)-C(9)-C(4)	112.09
C(4)-C(3)-C(10)	115.67	C(3)-C(10)-C(26)	114.32
C(3)-C(4)-C(5)	113.36	C(8)-C(13)-C(14)	124.94
C(5)-C(4)-C(9)	113.58	C(13)-C(14)-C(15)	123.91
C(4)-C(5)-C(6)	110.96	C(14)-C(15)-C(16)	112.54
C(4)-C(5)-C(11)	112.89	C(15)-C(16)-C(17)	110.80
C(6)-C(5)-C(11)	114.94	C(15)-C(16)-C(22)	110.48
C(5)-C(6)-C(7)	114.02	C(17)-C(16)-C(22)	109.57
C(5)-C(6)-C(12)	122.14	C(16)-C(17)-C(18)	120.32
C(7)-C(6)-C(12)	111.52	C(17)-C(18)-C(19)	106.26
C(6)-C(7)-C(8)	115.29	C(17)-C(18)-C(23)	111.35
O(2)-C(6)-C(7)	58.41	C(17)-(18)-O(3)	108.29
O(2)-C(7)-C(8)	112.62	C(19)-C(18)-C(23)	111.91
C(7)-C(8)-C(9)	110.94	C(18)-C(19)-C(20)	118.33
C(7)-C(8)-C(13)	107.87	C(19)-C(20)-C(21)	120.84
C(9)-C(8)-C(13)	116.80	C(9)-C(21)-C(20)	116.2

II. Bond lengths in (Å)

C(1)-N(2) = 1.337	C(8)-C(13) = 1.512
C(1)-O(1) = 1.229	C(9)-C(21) = 1.562
C(1)-C(9) = 1.515	C(10)-C(26) = 1.510
N(2)-C(3) = 1.447	C(13)-C(14) = 1.343
C(3)-C(4) = 1.552	C(14)-C(15) = 1.509
C(3)-C(10) = 1.539	C(15)-C(16) = 1.539
C(4)-C(5) = 1.550	C(16)-C(17) = 1.527
C(4)-C(9) = 1.575	C(16)-C(22) = 1.538
C(5)-C(6) = 1.524	C(17)-C(18) = 1.530
C(5)-C(11) = 1.539	C(18)-C(23) = 1.539
C(7)-C(8) = 1.530	C(18)-O(3) = 1.420
C(6)-O(2) = 1.436	C(18)-C(19) = 1.527
C(7)-O(2) = 1.437	C(19)-C(20) = 1.504
C(6)-C(7) = 1.507	C(19)-O(4) = 1.434
C(7)-C(8) = 1.530	C(20)-O(4) = 1.440
C(8)-C(9) = 1.550	C(20)-C(21) = 1.532

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