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# Examination of the Conformational Properties of the Prymnesin System: A Computational Chemistry Approach

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Prymnesins are potent ichthyotoxic and hemolytic glycoside molecules isolated from the red tide alga *Prymnesium parvum*. Their unique molecular structure is conformationally rich with a number of key torsional bonds which were probed with contemporary conformational searching techniques in order to describe the three-dimensional structure of the prymnesin molecule. Important topological features include a backbone twist of 60° and an elongation length of 39.5 Å from ring "A" to ring "N" for the prymnesin-2 molecule in the nOe preferred conformation. An appreciation of these and other molecular structural and energetic features determined by computational techniques were examined with the aim of understanding how prymnesin-2 exhibits it's potent biological activity in relation to these inherent structural traits.

The historical notoriety of the series of compounds collectively known as "prymnesins" has its foundations in their unusual skeletal structure, scarcity of available purified material, and possible mode(s) of biological action.[1] Only until recently has the precise planar structure of prymnesin-2, Fig. 1, been fully known,[2] revealing an aesthetically pleasing and functionally diverse marine natural product consisting of an electron-rich hydrophobic "*head*" section (C1 to C20), a polyether-polycyclic "*backbone*" with five interconnecting sigma bonds connecting the central four biheterocyclic moieties and a pentaheterocyclic system (C20 to C74), and a hydroxyl-rich hydrophilic glycoside-bearing "*tail*" section (C74 to C90).

Our group's interest in the applicability of contemporary computational chemistry techniques for use in the study of large biologically interesting organic compounds of marine origin was prompted by our continual pursuit of a thorough understanding of the modes of action and three-dimensional structures of these molecules. We envisaged that through such a study and in cross evaluation with available biological, chemical, and NMR studies that certain pertinent aspects with regard to molecular conformation and functional group positioning within a molecule could be evaluated and examined in light of a "constructive biologically active model" which could possibly be used to explain the topological and morphological regions or sites of interaction on receptors, channels, membranes, or binding sites. A rationale following a similar line of thought has already been purported for the investigation of an analogous series of compounds from marine origin.[3]

Reported in this paper is a detailed computational study and cross evaluation of the conformational properties of prymnesin-2 using a variety of contemporary molecular mechanics force fields, software, and conformational search routines with the aim of providing a molecular basis for understanding the pronounced ichthyotoxic and hemolytic activity of prymnesin-2.

## Computational Methods and Techniques

**Molecular Modeling.** All molecular modeling, structure calculations, and conformational analyses were performed on a Silicon Graphics Indigo XS24 R4000 workstation using Sybyl® v6.1a software.[4] The supplied Tripos force field[5] was used routinely with the Powell (conjugate gradient) method in energy minimizations and conformational runs. Minimizations were deemed to have converged when either a maximum of 5000 iterations were calculated or the gradient reached 0.01 $\mathrm{kcal\,mol^{-1}\,Å^{-1}}$. Netbatch[6] (as supplied) was used consistently for minimization and conformational experiments. Electrostatics were incorporated into minimization calculations by inclusion of atomic point charges from the Gasteiger and Marsili Charge Sets to the force field parameterization of the atoms.[7]

**Conformational Analysis Experiments.** Conformational experiments about acyclic sigma bonds were conducted by means of *torsionally driving* the bond in question. Typically, the following parameters were employed for conformational searching experiments of individual acyclic sigma bonds. Starting from a conformational minimum, the conformational space of any one sigma bond was examined from 0 to 360 degrees about the bond in question, at 2 degree increments, with energy minimization after each increment of all new torsionally derived conformers (180 conformers per run). For the "*backbone*" (rings "E" to "N" and "A" to "N") section, quintuplet acyclic sigma bond searching experiments using the following search criteria were utilized; each sigma bond of the "*backbone*" was probed from 60 to 300 degrees at 120 degree increments, with minimization of all new torsionally found conformers

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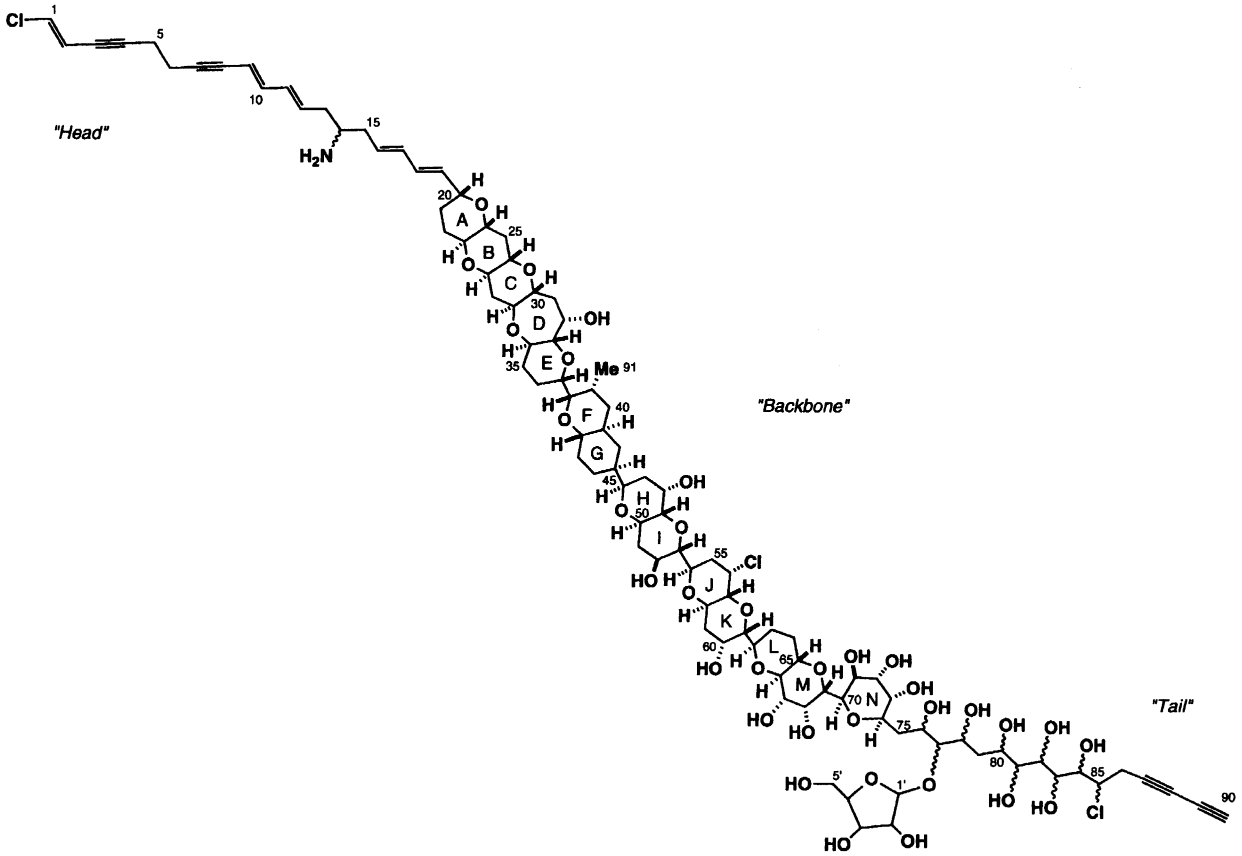

Fig. 1. Prymnesin-2, an example from the prymnesin series of molecules illustrating the conformational and functional group complexity of these molecules. The relative stereochemistry of the "*backbone*" section (C20 to C74) of the molecule was determined from nOe data.[2]

(243 conformers per experiment). The results were then analyzed by means of spreadsheet and graphical representations. Examination of the conformational space of the seven-membered "D" ring of the prymnesin system was accomplished by means of a *random torsional* searching technique.[8] Starting structural conformers for the seven-membered "D" ring were chosen intuitively and from examination of the literature,[3,8—10] with energy minimized prior to initiating the random searching routine. Random searches of the "D" ring were conducted with the following parameters and convergence criteria;[11] Maximum cycles 1000 to 1500, Energy Window (Cutoff) 50 to 70 kcal mol$^{-1}$, Convergence threshold (for uniqueness of conformers) 0.005 to 0.1 kcal mol$^{-1}$, with a maximum of 6 to 10 conformer repetitions.

**Other Molecular Mechanics Calculations.** Comparative computational studies were conducted using Insight® II (version 2.3.0) and Discover® (version 2.9.5 and 94.0)[12] with the CVFF (Consistent Valence force field)[13] available within the Biosym Technologies molecular modeling software. Charges were assigned using the potential function atom types and partial charges available within the software.[12] Minimizations were conducted using a conjugate gradient minimizer and were deemed to have converged when the gradient reached 0.001 kcal mol$^{-1}$ Å$^{-1}$ for all fragments investigated. Molecular mechanics minimization of fragments using the MM2(77) force field[14] were submitted as an internal submission from the "Sybyl" molecular modeling software to the netbatch system for processing. Analysis of the results was done externally to the "Sybyl" software. The MM2(77) experiment was deemed to have converged when the gradient reached 0.001 kcal mol$^{-1}$ Å$^{-1}$.

**Fragmentation of the Prymnesin System.** The prymnesin system was divided into conformationally interesting fragments and sections with the site of fragmentation (i.e. the termini of a fragment) being modified as follows. Where the joining atom to a bond cleavage site was a carbon atom, this carbon was replaced with a methyl group and for the cleavage of an ether bond, a hydroxyl group was substituted.

## Results and Discussion

The methodology used in this study of the conformational space of the prymnesin system was one of a 'pruning', 'computation', 'analysis', 'rebuild', 'analysis' approach. Such a technique was adopted in order to best use the available instrumental facilities and also allow reasonable experimental time frames to be followed.

To analyze the conformationally complex prymnesin system it was essential to initially simplify the system. The prymnesin molecule is characterized by a 14 heterocyclic ring "*backbone*" which links the predominantly unsaturated C19 "*head*" of the molecule to the C16 highly hydroxyl functionalized, sugar substituted "*tail*" of the molecule (Fig. 1). To examine the conformational space about the sigma bonds connecting the cyclic systems which constitute the prymnesin "*backbone*", from ring "A" to ring "N" (C20 to C74), the molecule was fragmented into discrete polycyclic systems which were individually conformationally searched.

These fragments were (with the torsional bond of interest in brackets); "E-F/G" [C36–C37–C38–C39], "F/G-H/I" [C44–C45–C46–C47], "H/I-J/K" [C52–C53–C54–C55], "J/K-L/M" [C60–C61–C62–C63], and "L/M-N" [C68–C69–C70–C71], with a methyl or hydroxyl group added in place of a connecting carbon atom or oxygen respectively, as shown in Chart 1. The pentaheterocyclic ring system from ring "A" to ring "E" (C20 to C37) containing the conformationally variable seven-membered "D" ring was examined as "C/D/E" and "A/B/C/D/E" fragments as illustrated in Chart 1.

**"E to N" Fragment Conformational Searching Experiments.** Conformational experiments about the interconnecting sigma bonds of the fragments, "E-F/G", "F/G-H/I","H/I-J/K", "J/K-L/M", and "L/M-N", were conducted by means of *torsionally driving* the bond in question. Typically, starting from a conformational minimum, the conformational space of any one sigma bond was examined from 0 to 360 degrees about that bond, at 2 degree increments, with energy minimization after each increment of all new torsionally derived conformers (180 conformers per run).

The resultant three lowest energy conformers found for each of the fragments investigated by the torsional driving experiments are compiled in Table 1. These lowest energy conformers have been described as either *anti* (*A*), positive *gauche* (+*G*), or negative *gauche* (−*G*) depending on their relative torsional angle, defined by the carbon chain, as illustrated in Fig. 2.

Table 1 lists for the three lowest energy conformers of

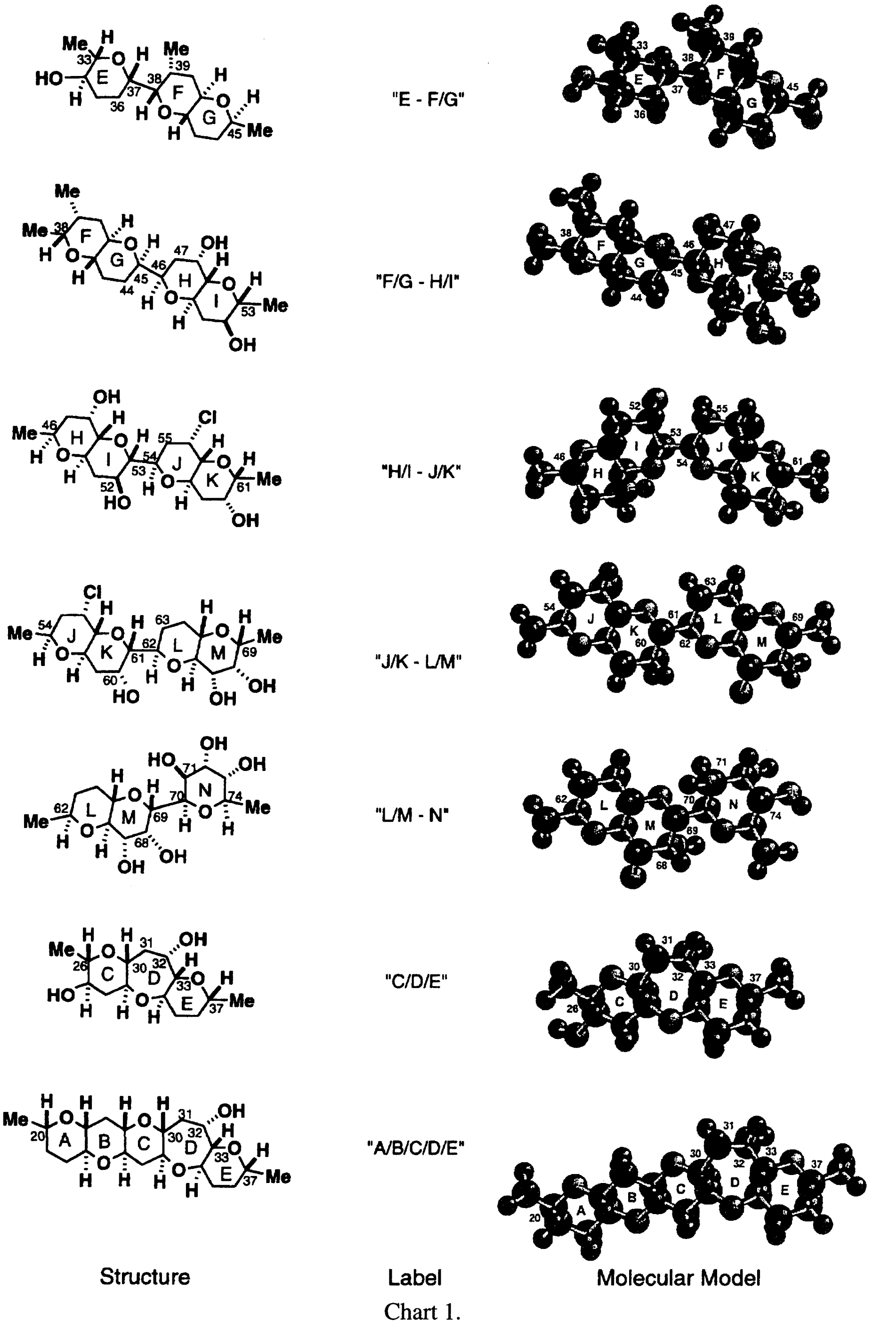

Chart 1.

Table 1. Comparison in Force Field Energies and Torsional Angles for Fragments of the Prymnesin System from "E to N" for the Tripos Force Field,[5] the MM2(77) Force Field,[14] and the CVFF Force Field,[13] Consisting of the Three Lowest Energy Conformers (*anti* (*A*) and two *gauche* (+*G* and −*G*))

| Fragment | Type | Tripos force field[5] (in vacuo) | | | | MM2(77) force field[14] (in vacuo) | | | | CVFF force field[13] (in vacuo) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Torsional angle No.) | | Total energy (kcal mol$^{-1}$) | Torsional angle (degrees) | Relative energy (kcal mol$^{-1}$) | Rank | Total energy (kcal mol$^{-1}$) | Torsional angle (degrees) | Relative energy (kcal mol$^{-1}$) | Rank | Total energy (kcal mol$^{-1}$) | Torsional angle (degrees) | Relative energy (kcal mol$^{-1}$) | Rank |
| E-F/G (C36–C37–C38–C39) | *A* | 7.7 | −171.4 | 0.0 | 1 | 40.6 | −172.1 | +0.6 | 3 | 25.7 | −165.9 | +1.5 | 3 |
| | +*G* | 8.2 | 93.6 | +0.5 | 3 | 40.1 | 91.1 | +0.1 | 2 | 24.9 | 82.7 | +0.7 | 2 |
| | −*G* | 8.1 | −55.9 | +0.4 | 2 | 40.0 | −53.6 | 0.0 | 1 | 24.2 | −55.3 | 0.0 | 1 |
| F/G-H/I (C44–C45–C46–C47) | *A* | 9.5 | −178.6 | +0.6 | 2 | 55.3 | −179.6 | +0.6 | 2 | 33.3 | 174.2 | +0.9 | 2 |
| | +*G* | 10.9 | 54.0 | +2.0 | 3 | 56.3 | 50.1 | +1.6 | 3 | 33.6 | 58.1 | +1.2 | 3 |
| | −*G* | 8.9 | −71.7 | 0.0 | 1 | 54.7 | −73.1 | 0.0 | 1 | 32.4 | −66.2 | 0.0 | 1 |
| H/I-J/K (C52–C53–C54–C55) | *A* | 9.8 | 174.0 | 0.0 | 1 | 62.0 | 174.7 | 0.0 | 1 | 12.6 | −178.8 | 0.0 | 1 |
| | +*G* | 10.9 | 72.0 | +1.1 | 2 | 64.0 | 74.0 | +2.0 | 2 | 16.8 | 69.3 | +4.2 | 2 |
| | −*G* | 11.8 | −70.5 | +2.0 | 3 | 65.2 | −73.6 | +3.2 | 3 | 17.0 | −71.5 | +4.4 | 3 |
| J/K-L/M (C60–C61–C62–C63) | *A* | 14.5 | −171.5 | 0.0 | 1 | 72.4 | −174.8 | 0.0 | 1 | 30.5 | −169.9 | +0.4 | 2 |
| | +*G* | 17.5 | 82.5 | +3.0 | 3 | 76.3 | 74.7 | +3.9 | 2 | 31.3 | 76.2 | +1.2 | 3 |
| | −*G* | 15.9 | −79.5 | +1.4 | 2 | 77.4 | −78.7 | +5.0 | 3 | 30.1 | −68.8 | 0.0 | 1 |
| L/M-N (C68–C69–C70–C71) | *A* | 18.9 | −179.9 | 0.0 | 1 | 79.3 | 179.5 | 0.0 | 1 | 28.8 | −179.5 | +3.1 | 3 |
| | +*G* | 20.5 | 97.0 | +1.6 | 3 | 81.3 | 91.0 | +2.0 | 2 | 25.7 | 64.8 | 0.0 | 1 |
| | −*G* | 19.3 | −85.6 | +0.4 | 2 | 84.9 | −71.0 | +5.6 | 3 | 27.3 | −74.2 | +1.6 | 2 |

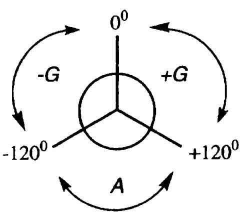

Fig. 2. The nomenclature used to assign the three lowest energy conformers found from torsional driving experiments, the *anti* (*A*) and two *gauche* (+*G* and −*G*) conformers.

each fragment, the minimized conformer energy and torsional angle for the three different force fields used in this study, namely; the Tripos force field,[5] the MM2(77) force field,[14] and the Consistent Valence force field (CVFF).[13] The relative energy and rank of each of the conformers for any one fragment have been computed with the 'global' minimum conformer having a rank of 1, and the next two 'local' minima conformers having a rank of 2 and 3, respectively.

Comparing the results obtained for the Tripos force field and the MM2(77) force field (Table 1), it can be seen that the two force fields show a high degree of similarity in the results for the lowest energy conformer, with the only variation occurring for the "E-F/G" fragment (Tripos *A*, whilst MM2 −*G*). The relative difference in energies between these two conformers for each of the force fields is 0.4 kcal mol$^{-1}$ (Tripos) and 0.6 kcal mol$^{-1}$ (MM2), which are comparably small. Conversely, comparing the Tripos force field with the Consistent Valence force field (Table 1), indicates only two fragments in which the lowest energy conformers have the same geometry for both the force fields, the "F/G-H/I" (−*G*) and the "H/I-J/K" (*A*) fragment. All other fragments of the "E to N" backbone of prymnesin show variations in the lowest energy conformer, significantly so for the "L/M-N" fragment with the Tripos force field returning the *A*-conformer as the lowest energy conformer and the CVFF the +*G*-conformer, with a 1.6 kcal mol$^{-1}$ and 3.1 kcal mol$^{-1}$ difference in energies for each of these force fields (respectively) between the two conformers ($\Delta E = |E_A - E_{+G}|$). These variations in energies may be due to the different parameterization of the atoms used by these two force fields. To the credit of all three force fields examined, the variation in the three lowest energy conformations (*A*, +*G*, and −*G*) within any force field evaluation was comparably consistent and the variation between different members of the lowest energy conformers was very small. An appreciation of the "closeness" in the obtained results is illustrated in Table 2. Finally, comparing the MM2(77) force field with the Consistent Valence force field, shows that there was moderate to good agreement between the lowest energy conformers returned for both these force fields, however discrepancies do occur (see fragments "J/K-L/M" and "L/M-N").

The results described above can be summarized and related to the geometry of the nOe preferred "E to N" section of the prymnesin system.[2] Table 2 draws together the results of the multi force field examination. It can be seen that the Tripos force field bears the greatest similarity to the

Table 2. Summary of Force Field Results for the Five "E to N" Fragments of the Prymnesin System

| Fragement | nOe | Tripos force field[5] | | MM2(77) force field[14] | | CVFF force field[13] | |
|---|---|---|---|---|---|---|---|
| | preferred | Lowest | $\leq$+1.0 kcal[a] | Lowest | $\leq$+1.0 kcal[a] | Lowest | $\leq$+1.0 kcal[a] |
| E-F/G | *A* | *A* | +*G*&−*G* | −*G* | *A*&+*G* | −*G* | +*G* |
| F/G-H/I | *A* | −*G* | *A* | −*G* | *A* | −*G* | *A* |
| H/I-J/K | +*G* | *A* | — | *A* | — | *A* | — |
| J/K-L/M | *A* | *A* | — | *A* | — | −*G* | *A* |
| L/M-N | *A* | *A* | −*G* | *A* | — | +*G* | — |

a) The next lowest energy conformers found within approximately $\leq$+1.0 kcal mol$^{-1}$ in energy for the Tripos,[5] MM2(77),[14] and CVFF[13] force fields.

preferred nOe geometry (varying only in the "F/G-H/I" and "H/I-J/K" fragment geometries), with the MM2(77) force field varying in three fragment geometries and the CVFF varying in all fragment geometries. To a first approximation (within 1.0 kcal mol$^{-1}$ of the lowest (global minimum) energy conformation) both the Tripos and MM2(77) force fields show the greatest similarity with the nOe preferred geometries (varying only in the "H/I-J/K" fragment geometry) whilst the Consistent Valence force field still shows a large degree of variation when the results are interpolated to a first approximation.

**"D" Ring Conformational Searching Experiments.** To probe the conformational space of cyclic molecules, there are currently a number of competitive techniques which can be used.[15] In this study, the conformationally interesting "D" ring of the prymnesin molecule was examined using a *random torsional* searching technique.[8] Although most methods currently available have been designed and tested only on simple (symmetric) cyclic alkanes, recent studies on heteroatom-bearing cyclic molecules have given credibility to these techniques for searching heteroatom, asymmetric, poly-hybridized cyclic systems.[3,16] We have nevertheless approached the study of ring conformational flexibility in polyether-polycyclic molecules with a degree of caution.

Random torsional searching experiments of the seven-membered "D" ring were performed on the "C/D/E" and "A/B/C/D/E" fragments (Chart 1), with the starting conformations of the "D" ring chosen intuitively and from examination of the literature.[3,10,16] Cycling experiments to either a maximum of 1000 or 1500 random increments or 6 to 10 conformer repetitions for a variety of starting conformations revealed two unique lowest energy conformers for the seven-membered "D" ring. These conformers were arbitrarily labeled as the 'nOe preferred' conformer (with a intracyclic (C30–C31–C32–C33) dihedral angle of approximately −40°, which corresponds to the preferred conformer as determined by nOe experiments[2]) and the 'Compt. derived' conformer (with an intracyclic (C30–C31–C32–C33) dihedral angle of approximately +40°) (Fig. 3).

Table 3 brings together the minimized energy and torsional angle data for the two lowest energy conformers of the "D" ring for the three force fields used in this study. In general, all the force fields examined reflected similar trends, in that the 'nOe preferred' conformer has consistently the lower energy of the two minimum energy conformers. Likewise, the dihedral (C30–C31–C32–C33) angles have similar magnitudes of deflection from the eclipsed 0° conformer of approximately ±40° for the 'nOe preferred' and 'Compt. derived' conformers when calculated with the Tripos and MM2(77) force fields, whilst with the Consistent Valence force field,

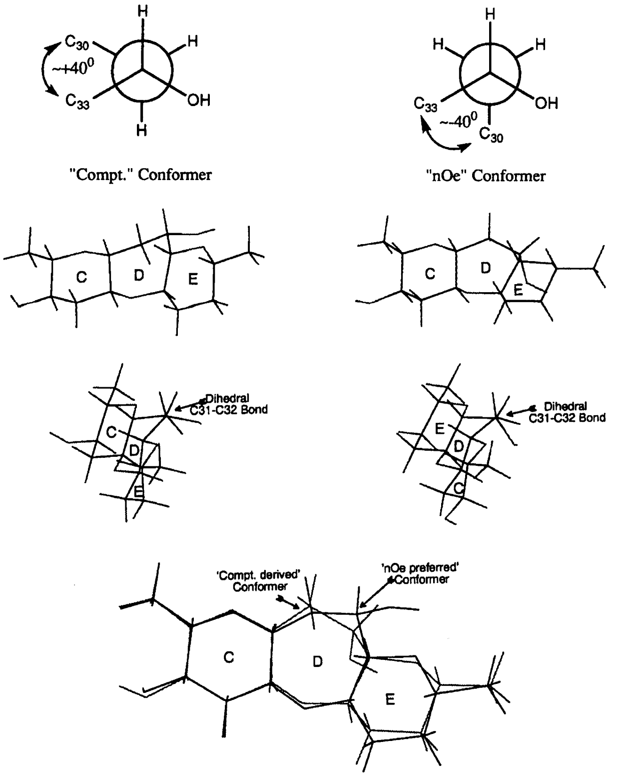

Fig. 3. Newman projections of the "D" ring carbon bond bridge between rings "C" (C30) and "E" (C33) which subtends the dihedral angle, C30–C31–C32–C33 (top), the corresponding molecular models for the "C/D/E" fragment (middle) and an overlay of the two lowest energy "D" ring conformers for the "C/D/E" fragment. Illustrated are the 'nOe preferred' conformer (RHS) and the 'Comput. derived' conformer (LHS) as determined by random torsional searching experiments.

Table 3. Comparison in Force Field Energies and "D" Ring Dihedral Angles for Fragments of the Prymnesin System "C/D/E" and "A/B/C/D/E" with the Tripos Force Field,[5] the MM2(77) Force Field,[14] and the CVFF Force Field,[13] Consisting of the Two Lowest Energy "D" Ring Conformers (the 'nOe preferred' and the 'Compt. derived')

| Fragment (Carbon chain numbering) | Tripos force field[5] (in vacuo) | | MM2(77) force field[14] (in vacuo) | | CVFF force field[13] (in vacuo) | |
|---|---|---|---|---|---|---|
| | Energy (kcal/mol) | Dihedral angle[a] (degrees) | Energy (kcal/mol) | Dihedral angle[a] (degrees) | Energy (kcal/mol) | Dihedral angle[a] (degrees) |
| "C/D/E" nOe (C26 to C37) | 15.2 | −43.1 | 47.1 | −41.6 | 14.2 | −28.2 |
| "C/D/E" Compt. (C26 to C37) | 17.5 | 35.3 | 48.0 | 35.1 | 18.0 | 27.7 |
| "A/B/C/D/E" nOe (C20 to C37) | 13.8 | −42.6 | 58.0 | −40.4 | 25.1 | −28.1 |
| "A/B/C/D/E" Compt. (C20 to C37) | 17.0 | 34.7 | 59.3 | 33.1 | 29.4 | 21.8 |

a) The dihedral angle of the "D" ring is the angle subtended by the bonds C30–C31–C32–C33.

the dihedral angle was found to be consistently more acute (±20—30°). In separate experiments on the "C/D/E" fragment, it was possible to estimate the energy of activation for interconversion of the two conformers to be approximately 1.6 kcal mol$^{-1}$.[17]

**Conformational Searching of the Prymnesin "*Backbone*" (C20 to C74).** Compiling the results of the experiments performed above, involving discrete fragments of the prymnesin "*backbone*" bearing only one conformationally flexible region, into a more conformationally demanding experiment, the prymnesin "*backbone*", rings "A" to "N" and "E" to "N", were used to further study and appreciate the conformational properties of the prymnesin system, particularly with reference to gaining an understanding of the energy and conformation of the 'global' "*backbone*" minimum in relation to other 'local' "*backbone*" minima conformers.

The "E to N" fragment of the prymnesin system which has five unique interconnecting sigma bonds connecting the polyether-polycyclic systems theoretically has an infinite number of rotatable conformers obtainable about these bonds (and combinations thereof). However, if we make the assumption that about these bonds the ground state conformer lies in one of the three possible minimum energy conformations of either *gauche* (two possible conformers, +*G* or −*G*) or *anti* (one possible conformer, *A*) (vide supra), then there are 243 low energy conformers for the "E to N" fragment.[18] Realistically, given the size and complexity of the prymnesin "*backbone*" 243 possible minimum energy conformers are a large but attainable conformational space problem. Thus, a pervading way to address and understand such a complex conformational problem is by performing a quintuplet torsional searching experiment, which targets each of the three low energy regions consisting of the five sigma bonds of the "E to N" and "A to N" "*backbone*" fragments. Having done so, energetic and geometric relationships between the nOe preferred and the computationally predicted 'global' minimum conformer can be evaluated. Table 4 compiles the results of these conformational searching experiments listing the 30 lowest energy conformer combinations and the highest energy conformer combination for torsional searches of both the "E to N" and "A to N" "*backbone*" fragments (Tripos force field).

The minimized nOe preferred conformer can be described as having an *A*/*A*/+*G*/*A*/*A* torsional chain "E to N" and "A to N" "*backbone*" system. Alternatively, a "favored" conformer for the "E to N" and "A to N" fragment is *A*/−*G*/*A*/*A*/+*G*, Table 4. This bears a degree of resemblance to the results obtained for the individual sigma bond searching experiments described above (particularly for the torsional bonds from the "E" to the "L" rings) where an *A*/−*G*/*A*/*A*/*A* geometry is predicted (Table 1, Tripos force field). Interestingly and significantly, the "*A*" and "+*G*" conformers for the C68–C69–C70–C71 bond of the "L/M-N" fragment differ in energy by only 1.6 kcal mol$^{-1}$ (Tripos force field, Table 1).[19]

From Table 4, the nOe preferred conformation of the prymnesin "*backbone*" in both the "E to N" and "A to N" fragments lie within 2.7 kcal mol$^{-1}$ of the 'global' minimum conformation and are the estimated 24 and 25 ranked lowest energy conformer combinations for each "*backbone*" section investigated. The conformer combination predicted from individual sigma bond fragment searches (Tripos force field) are within 1.9 kcal mol$^{-1}$ of the 'global' minimum conformer combination and are ranked 16 of the lowest energy conformer combinations for both fragment systems. Interestingly, the nOe preferred conformer combination is 5.8 kcal mol$^{-1}$ and 6.0 kcal mol$^{-1}$ more stable than the highest +*G*/+*G*/−*G*/+*G*/−*G* conformer combination for the "E to N" and "A to N" fragment systems, respectively.

**"C1 to C20" Fragment Conformational Searching.** The "*head*" section of the prymnesin molecule, that is "C1 to C20", has conceivably three structurally rigid sections, due to distinct π-systems; C1 to C5, C6 to C13, and C15 to C20, with many degrees of free rotation about the C5–C6, C13–C14 and C14–C15 (sp$^3$–sp$^3$) σ-bonds and C12–C13, C15–C16 and C19–C20 (sp$^2$–sp$^3$) σ-bonds. The intrinsic stability of the two π-systems defined by the transoid conjugated butadiene systems C9–C12 and C16–C19 lies in

Table 4. Tabulated Results from the Quintuplet Acyclic Sigma-Bond Search of the "*Backbone*" Section of the Prymnesin Molecule for Rings "A to N" and "E to N"

| "A to N" | | | | | | | "E to N" | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rank | Relative energy[a] (kcal mol$^{-1}$) | E-F/G (C36–C37–C38–C39) | F/G-H/I (C44–C45–C46–C47) | H/I-J/K (C52–C53–C54–C55) | J/K-L/M (C60–C61–C62–C63) | L/M-N (C68–C69–C70–C71) | Rank | Realtive energy[a] (kcal mol$^{-1}$) | E-F/G (C36–C37–C38–C39) | F/G-H/I (C44–C45–C46–C47) | H/I-J/K (C52–C53–C54–C55) | J/K-L/M (C60–C61–C62–C63) | L/M-N (C68–C69–C70–C71) |
| 1 | 0.0 | *A* | −*G* | *A* | *A* | +*G* | 1 | 0.0 | *A* | −*G* | *A* | *A* | +*G* |
| 2 | 0.1 | −*G* | −*G* | *A* | *A* | +*G* | 2 | 0.3 | −*G* | −*G* | *A* | *A* | +*G* |
| 3 | 0.3 | *A* | −*G* | +*G* | *A* | +*G* | 3 | 0.4 | *A* | −*G* | +*G* | *A* | +*G* |
| 4 | 0.5 | −*G* | −*G* | +*G* | *A* | +*G* | 4 | 0.7 | *A* | *A* | *A* | *A* | +*G* |
| 5 | 0.7 | *A* | *A* | *A* | *A* | +*G* | 4 | 0.7 | −*G* | −*G* | +*G* | *A* | +*G* |
| 6 | 0.8 | −*G* | *A* | *A* | *A* | +*G* | 6 | 0.9 | *A* | −*G* | −*G* | *A* | +*G* |
| 6 | 0.8 | *A* | −*G* | *A* | −*G* | +*G* | 6 | 0.9 | *A* | −*G* | *A* | −*G* | +*G* |
| 8 | 0.9 | *A* | −*G* | −*G* | *A* | +*G* | 8 | 1.0 | −*G* | *A* | *A* | *A* | +*G* |
| 9 | 1.1 | *A* | *A* | +*G* | *A* | +*G* | 8 | 1.0 | *A* | *A* | +*G* | *A* | +*G* |
| 10 | 1.2 | −*G* | −*G* | −*G* | *A* | +*G* | 10 | 1.2 | −*G* | −*G* | −*G* | *A* | +*G* |
| 11 | 1.4 | −*G* | *A* | +*G* | *A* | +*G* | 10 | 1.2 | −*G* | −*G* | *A* | −*G* | +*G* |
| 11 | 1.4 | *A* | *A* | *A* | −*G* | +*G* | 12 | 1.4 | *A* | *A* | *A* | −*G* | +*G* |
| 13 | 1.5 | *A* | *A* | −*G* | *A* | +*G* | 12 | 1.4 | −*G* | *A* | +*G* | *A* | +*G* |
| 14 | 1.6 | −*G* | *A* | *A* | −*G* | +*G* | 14 | 1.5 | *A* | *A* | −*G* | *A* | +*G* |
| 15 | 1.8 | −*G* | −*G* | *A* | −*G* | +*G* | 15 | 1.8 | −*G* | *A* | *A* | −*G* | +*G* |
| 16 | 1.9 | *A* | −*G* | *A* | *A* | *A* | 16 | 1.9 | *A* | −*G* | *A* | *A* | *A* |
| 16 | 1.9 | *A* | +*G* | *A* | *A* | +*G* | 16 | 1.9 | −*G* | *A* | −*G* | *A* | +*G* |
| 18 | 2.0 | −*G* | −*G* | *A* | *A* | *A* | 18 | 2.0 | *A* | +*G* | *A* | *A* | +*G* |
| 19 | 2.1 | −*G* | +*G* | *A* | *A* | +*G* | 19 | 2.2 | *A* | −*G* | +*G* | *A* | *A* |
| 20 | 2.2 | *A* | −*G* | +*G* | *A* | *A* | 20 | 2.3 | −*G* | −*G* | *A* | *A* | *A* |
| 21 | 2.4 | −*G* | −*G* | +*G* | *A* | *A* | 20 | 2.3 | −*G* | +*G* | *A* | *A* | +*G* |
| 22 | 2.5 | *A* | *A* | *A* | *A* | *A* | 22 | 2.5 | *A* | *A* | *A* | *A* | *A* |
| 23 | 2.6 | −*G* | *A* | −*G* | *A* | +*G* | 23 | 2.6 | −*G* | −*G* | +*G* | *A* | *A* |
| 23 | 2.6 | −*G* | *A* | *A* | *A* | *A* | 24[b] | 2.7 | *A* | *A* | +*G* | *A* | *A* |
| 25[b] | 2.7 | *A* | *A* | +*G* | *A* | *A* | 25 | 2.8 | −*G* | *A* | *A* | *A* | *A* |
| 26 | 2.8 | *A* | −*G* | −*G* | *A* | *A* | 25 | 2.8 | *A* | −*G* | −*G* | *A* | *A* |
| 27 | 3.1 | −*G* | *A* | +*G* | *A* | *A* | 27 | 3.1 | −*G* | −*G* | −*G* | *A* | *A* |
| 27 | 3.1 | −*G* | −*G* | −*G* | *A* | *A* | 28 | 3.3 | −*G* | *A* | +*G* | *A* | *A* |
| 29 | 3.4 | *A* | *A* | −*G* | *A* | *A* | 29 | 3.4 | *A* | *A* | −*G* | *A* | *A* |
| 30 | 3.5 | −*G* | *A* | −*G* | *A* | *A* | 30 | 3.7 | −*G* | *A* | −*G* | *A* | *A* |
| 243 | 8.7 | +*G* | +*G* | −*G* | +*G* | −*G* | 243 | 8.5 | +*G* | +*G* | −*G* | +*G* | −*G* |

a) Relative energy refers to evaluation with the Tripos force field, in vacuo. b) Indicates the nOe preferred *A*/*A*/+*G*/*A*/*A* conformer.

the unoccupied antibonding π-orbitals of the sp$^2$-carbons (between C10–C11 and C17–18), and is a well-founded assertion in organic chemistry.20) The acetylenic groups also impart molecular rigidity into this section of the molecule with the four sp-hybridized carbons C3, C4, C7, and C8 asserting linearity to the molecule from C2 to C5 and C6 to C9. Combining these properties with the (*E*)-olefinic C1–C2 bond and the nOe-derived orientation at the ring contact atom C20, implies that the "C1 to C2" portion of the prymnesin system has essentially three primary sites of conformational flexibility about sp$^3$–sp$^3$ σ-bonds, at C5–C6, C13–C14, and C14–C15 and three primary sites of conformational flexibility about sp$^2$–sp$^3$ σ-bonds, at C12–C13, C15–C16, and C19–C20.

Evaluating the conformational space of the sp$^3$–sp$^3$ σ-bonds initially, a dual torsional searching experiment was performed on the C13–C14/C14–C15 section of the "C1 to C20" fragment (C20 being replaced with a methyl group), and as expected returned nine minimum energy conformers. These conformations correspond to combinations of the low energy *anti* and two *gauche* conformers for the C13–C14 and C14–C15 bonds. All the lowest energy conformations were of roughly equal energy and thus share equal probability of being occupied by the lowest energy (global minimum) conformer. Similarly, the C5–C6 bond was found to have the staggered conformations, *anti* and two *gauche* conformers as the three lowest energy conformers.

Interestingly, torsional searching of the sp$^2$–sp$^3$ σ-bonds C12–C13, C15–C16, and C19–C20 revealed intriguing structural traits for these rotatable bonds. In all the cases examined, there were two lowest energy (global) minima energy conformers found which were diequally opposed (at a torsional angle of approximately ±120°) about the other lowest energy conformer (at a torsional angle of 0°). These torsional angles impart geometries about these bonds in which the carbon olefinic chain and also the coplanar protons of the olefinic sp$^2$-carbons are all quasi-planar with (or eclipsing one thereof) one of the substitutes of the sp$^3$-hybridized carbon. These were the only minimum energy conformers found for the sp$^2$–sp$^3$ σ-bonds.

Thus, each of the primary sites of rotation would essentially adopt either one of only three low energy conformational orientations, *anti* or one of two *gauche* orientations (sp$^3$–sp$^3$), or one of two diequally opposed positions (sp$^2$–sp$^3$) in a ground state biological system. Combining these results with the perceived free and rapid rotation about the sp–sp$^3$ σ-bonds (C4–C5 and C6–C7) suggests that the "*head*" section of the prymnesin molecule has many possible low energy conformations and degrees of spatial orientation.

**Conformational Searching of the Prymnesin "*Head*" (C1 to C20).** An appreciation of the conformational space occupied by the "*head*" section of the prymnesin molecule was accomplished by systematic torsional searching of the fragments "C1 to C20", "C1 to C25", and "C1 to C38" at all the key conformationally accessible and flexible regions as already described, vide supra. The lowest energy conformations for all the "*head*" fragments investigated were found to exhibit an interesting structural property, in that the diene systems, C9 to C12 and C16 to C19, adopted co-aligned parallel orientations with the H12–H16, H11–H17, H10–H18, and H9–H19 distance varying from 2.7 to 3.6 Å, as depicted in Fig. 4 for the "C1 to C38" fragment molecular model. Given the relative intra-molecular distances between the hydrogens of the diene systems and the lack of nOe enhancements between these nuclei,2) this energetically weak π-stacking or π-adduct conformation21) for these "*head*" section diene systems would presumably bear little resemblance to the expected solution conformation.22) The next lowest energy conformation for the "*head*" section in which this stabilizing π-stacking conformation is not present is an elongated "*head*" section, which is approximately 5.0 kcal mol$^{-1}$ higher in energy (Tripos force field) (Fig. 4).

**The "C74 to C90" "*Tail*" Section of Prymnesin.** The "*tail*" section, "C74 to C90", of the prymnesin molecule was not investigated with the computational techniques used in this study. This hydroxyl group-fertile, glycoside-bearing section of the molecule is conceivably very motile, with an abundance of low-energy conformer geometries and interconversion pathways. Also, at present, the stereochemistry at each of the nine chiral centers is still unknown. Conceivably, in the absence of a protic or donor solvent, a hydrogen bonding network may stabilize this section of the molecule imparting some rigidity via a secondary electrostatic frame-

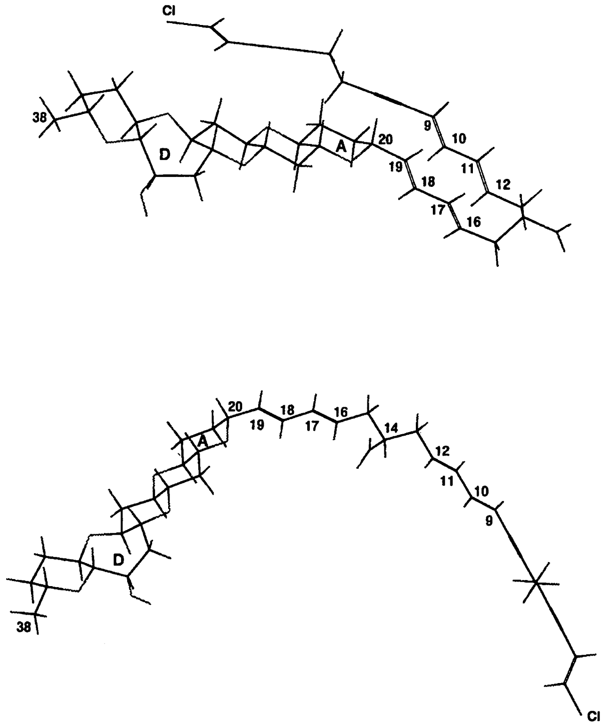

Fig. 4. "C1 to C38" fragment minimized molecular models (Tripos force field, in vacuo) illustrating (top) an example lowest energy π-stacking of the diene groups conformation, with the H12–H16, H11–H17, H10–H18, H9–H19 distance varying from 2.7 to 3.6 Å, and (bottom) an example from the 5.0 kcal mol$^{-1}$ more destabilized conformation, exhibiting an elongated "*head*" extension (from C1 to C20).

work. It can also be noted that this hydrophilic region of the molecule may be of pivotal importance with regard to the nature and mode of the association prymnesin has with membranes.

**Reconstruction of the "E to N" and "A to N" nOe Preferred Prymnesin "*Backbone*".** The conformational searching techniques and results described above for fragments of the prymnesin backbone represent a meticulous appraisal of the conformationally fertile prymnesin backbone and suggest that the conformation of the prymnesin backbone indicated by NMR data compares well with all three force fields used in this study. Table 5 compiles the data for the reconstructed prymnesin backbone "E to N" and "A to N", with the conformationally flexible regions of the "*backbone*" bearing the orientations analogously found from NMR experiments.[2)]

By appreciating planes which lie in the "seat" section of the chair conformation for each of the cyclic systems found at the termini of the backbone, it is possible to mathematically describe the conformational space of the prymnesin backbone further.[23)] For example, defining a plane which runs through the C20, C21, C23, and C24 atoms (ring "A") and measuring a normal to this plane which intersects the C74 atom at the "N" ring terminus of the prymnesin system suggests that the axial twist of the backbone, when viewed along the axis from ring "A" to ring "N" has deviated by approximately 4.0 Å from the "A" ring plane.[24)] Similarly, describing a plane which passes through the C70, C71, C73, and C74 atoms (ring "N") and measuring the angle of intersection with the "A" ring plane infers that the axial twist of the prymnesin backbone "A to N" is approximately one sixth of a circle or approximately 60° (Fig. 5). This "screwing" or "twisting" effect is a common secondary structural trait of large biological molecules as they seek to attain a minimum energy, maximum entropy steady state conformation.[25)] Such a second order structural characteristic may be accentuated further to the conformationally freer "*head*" and "*tail*" regions of the prymnesin system and indeed may be of particular importance, along with the type and position of the functional moieties of the prymnesin system, to understanding the possible biologically active forms or conformations of prymnesin. Structural inference with respect to biological activity is a well-founded mechanistic principle in bioorganic chemistry and similar suppositions with respect to the type of binding and structural characteristics of analogous polyether-polycyclic systems have already been presented in the literature.[26)]

## Conclusion

The conformationally plentiful prymnesin system has been examined computationally to reveal a backbone system "A to N" abundant with energetically discrete and definable minimum energy conformers. Principally, these local energy minima can be described as comprising the three low energy *anti*

Table 5. Reconstruction of the Prymnesin Backbone
The Polyether-Polycyclic Region for Ring Systems "E to N" and "A to N" in the 'nOe Preferred' Orientation, Minimized Energies and Geometrical Characteristic Features
(All data derived from the Tripos force field and Sybyl® V6.1a software.)

| Ring(s) | Dihedral/Torsional bonds | "E to N" | "A to N" |
|---|---|---|---|
| D[a)](degrees) | C30–C31–C32–C33 | — | −46.2 |
| E-F/G[a)](degrees) | C36–C37–C38–C39 | −171.5 | −172.2 |
| F/G-H/I[a)](degrees) | C44–C45–C46–C47 | −179.2 | −179.6 |
| H/I-J/K[a)](degrees) | C52–C53–C54–C55 | 71.0 | 70.6 |
| J/K-L/M[a)](degrees) | C60–C61–C62–C63 | −172.3 | −172.5 |
| L/M-N[a)](degrees) | C68–C69–C70–C71 | 179.4 | 179.4 |
| Fragment characteristics | | | |
| Total energy (kcal $mol^{-1}$) | — | 41.4 | 54.1 |
| Elongation length[b)] (Å) | — | 29.7 | 39.6 |
| Magnitude of backbone twist[c)](degrees) | — | 97.3 | 64.4 |
| Height of C74 above"E"ring plane[e)] (Å) | — | 9.9 | — |
| Height of C74 above"A"ring plane[d)] (Å) | — | — | 4.0 |
| Height of C33 above"N"ring plane[f)] (Å) | — | 11.0 | 11.1 |
| Height of C20 above"N"ring plane[f)] (Å) | — | — | 17.5 |

a) The "D" ring conformation and the conformation of all the polyether-polycyclic interconnecting sigma bonds are those conformations adopted by the nOe derived structure. b) "Elongation length" is defined as the straight line distance between the two termini at each end of the fragment (ie. from C33 to C74 for the "E to N" fragment or from C20 to C74 for the "A to N" fragment). c) "Backbone Twist" can be defined as the angle subtended by the two planes formed by the "seat" section of the chair conformers for the rings found on the extremities of the fragments (between rings "A" and "N" or "E" and "N", respectively). d) The "A" ring plane is defined as the plane which passes through the "seat" section of the chair conformer of this ring and thus which passes through the atoms, C20, C21, C23, and C24.[23)] e) The "E" ring plane is defined as the plane which passes through the "seat" section of the chair conformer of this ring and thus which passes through the atoms, C33, C34, C36, and C37.[23)] f) The "N" ring plane is defined as the plane which passes through the "seat" section of the chair conformer of this ring and thus which passes through the atoms C70, C71, C73, and C74.[23)]

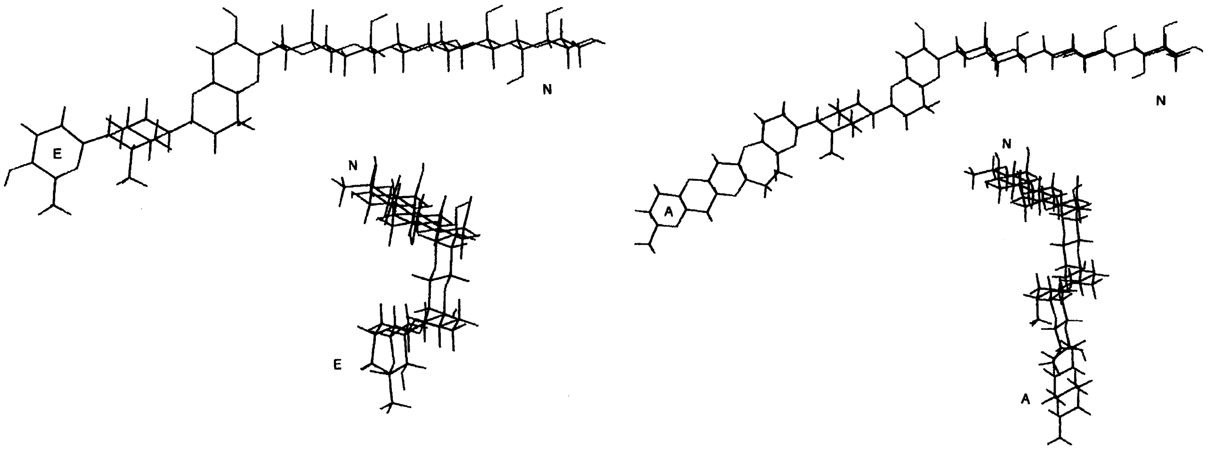

Fig. 5. Energy minimized (Tripos force field) molecular models of the "E to N" (LHS) and "A to N" (RHS) reconstructed prymnesin "*backbone*" in the nOe preferred orientation illustrating the intramolecular "backbone" 'twist' of the "E to N" fragment and the "A to N" fragment.

and two *gauche* conformers which occur for the torsional interconnecting $sp^3$–$sp^3$ $\sigma$-bonds between the polyether-polycyclic moieties and the two ring conformations of the seven-membered "D" ring which differ by approximately −40° to +40° about the dihedral C31–C32 bond and are comparable in energy.

Structurally, the prymnesin backbone in the nOe preferred conformation possesses an "intramolecular-twist" of approximately 60° from true and has an intramolecular elongation length of approximately 39.5 Å, for the "A to N" section of the prymnesin system.

Through this computational study of the conformational space of prymnesin-2, it has been possible to energetically understand, describe and visualize the three-dimensional structure of the prymnesin molecule. In light of this computational study which clearly examines the interplay of conformationally flexible regions with structurally rigid sections, it becomes apparent that the prymnesin molecule possesses three important regions for bioactivity, these being the "*head*", "*backbone*", and "*tail*" regions. The structural and energetic results presented here are currently being cross examined with coincidentally achieved and previously published biological activity data with the aim of understanding how the positioning of functional groups within the molecule, the *in vivo* and *in vitro* forms of the molecule, and the possible molecule/receptor interactive form of the molecule, produce the pronounced potent biological activity exhibited by these molecules. Therefore, this study serves as a starting point for i) our further investigation of the mechanism and relationships between the molecular structure of prymnesins and their pronounced ichthyotoxicity and hemolytic activity, and ii) the study of the molecular dynamics and interplay of conformational energetics of the prymnesin system, which are concurrently underway in our laboratory.

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11) Parameters apply to the "D" ring random torsionally searched in this study as part of the fragments "C/D/E" and "A/B/C/D/E" as described in the text.

12) Insight® II (Version 2.3.0) and Discover® (Version 2.9.5 and 94.0) are distributed by Biosym Technologies Inc., 9685 Scanton Road, San Diego, CA 92121-4778, USA.

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18) The theoretical number of conformers ($N$) is given by the expression; $N=(360/\Delta)^n$; where, $\Delta$ = the rotational increment (in degrees)= 120; and, $n$ = the number of rotatable bonds=5; thus, $(360/120)^5=3^5=243$ low energy conformers for the "E to N" backbone.

19) The barrier to rotation from the $+G$ or $-G$ conformer to the $A$ conformer has been calculated (Tripos force field) to be approximately, 5.0 kcal mol$^{-1}$, which is much lower than the 11.9 kcal mol$^{-1}$ barrier to rotation between the $+G$ and $-G$ conformers, clearly indicating that there is a lower energy pathway between the $+G$ and $A$ conformers (and vice versa).

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23) The mathematical formulae which describe the planes discussed in the text to this paper and referred to in Table 5 and Ref. 24 are as follows: The plane which passes through the "seat" section of the "A" ring and is defined by the atoms C20, C21, C23 and C24, can be described by the equation: $7.133e-02x -8.805e-01y -4.687e-01z -2.275e+01=0$ (RMS distance of defining atoms from plane = ±0.01 Å) for the "A to N" energy minimized fragment. The plane which passes through the "seat" section of the "E" ring and is defined by the atoms C33, C34, C36, and C37, can be described by the equation: $5.917e-01x -6.610e-01y -4.616e-01z -2.268e+01=0$ (RMS distance of defining atoms from plane = ±0.004 Å) for the "E to N" energy minimized fragment. The plane which passes through the "seat" section of the "N" ring and is defined by the atoms C70, C71, C73, and C74, can be described by the equations: $-8.653e-01x -4.746e-01y -1.612e-01z -1.462e+01=0$ (RMS distance of defining atoms from plane = ±0.0008 Å) for the "A to N" energy minimized fragment. $-8.667e-01x -4.740e-01y -1.553e-01z -1.449e+01=0$ (RMS distance of defining atoms from plane = ±0.0009 Å) for the "E to N" energy minimized fragment.

24) Similarly, describing a plane which passes through the C70, C71, C73, and C74 atoms (ring "N") and measuring a normal to this plane which intersects the C20 atom at the "A" ring terminus of the prymnesin system suggests that the axial twist of the backbone, when viewed along the axis from ring "N" to ring "A", has deviated by approximately 17.0 Å from the "N" ring plane, implying the angular twist along the backbone is not uniform in two dimensions, i.e. the resultant "screw" secondary structure of the backbone has deviated from the true "cylindrical" axis of rotation along the prymnesin backbone "A to N" to a "conical" axis of rotation.

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