Table V. Parameters Used in the Extended-Hückel Calculations

| atom | orbital | $H_{ii}$/eV | $\zeta_1$ | $\zeta_2$ | $C_1{}^a$ | $C_2{}^a$ |
|---|---|---|---|---|---|---|
| Nb | 5s | −10.10 | 1.89 | | | |
| | 5p | −6.86 | 1.85 | | | |
| | 4d | −12.10 | 4.08 | 1.64 | 0.6401 | 0.5516 |
| O | 2s | −32.30 | 2.275 | | | |
| | 2p | −14.80 | 2.275 | | | |

[a] Coefficients used in the double-ζ expansion of the 4d orbitals.

electronegative atoms.[27] The other advantage of this position is that they would not interfere with the bonding about Nb1. It is unlikely that the highly symmetrical coordination of this metal will incorporate a strange atom.

Do we have a triple bond between the paired Nb1's in this structure, as it has been described? First let us look at some of the bond length systematics. In the complex $[NEt_4]_2[Nb_2Cl_6(\mu\text{-}THT)_3]\cdot CH_3CN$ the metal–metal bond length is 263.2 pm. The bonding between the two formally $Nb^{2+}$, $d^3$, has been described as being a triple bond.[19b] Still slightly shorter Nb–Nb bonds are found in the tetramers $[Na(THF)_3]_2[Nb_2X_5(THT)_3]_2$, respectively 261.0 pm for X = Cl and 260.7 pm for X = Br, each unit containing two Nb–Nb triple bonds separated by a long Nb···Nb distance.[19b] In the molecular complexes $Cs_3Nb_2Cl_9$,[17] $[Nb_2(CO)_2(Cp)_2(C_2R_2)]$,[18] and $Nb_2Cl_6(depe)$,[19a] the niobium–niobium bond lengths are in the range 270–274 pm. They are thought to be double bonds as the two metals are formally $Nb^{3+}$, $d^2$. The longer bonds in the solids are closer to being single bonds.

If we examine the orbitals of our delocalized three-dimensional structure, we find that three of the four bands occupied are primarily localized on Nb1. Furthermore they have the shape of **18**, **19**, and **20**. While there is some admixture of other orbitals, it is clear that these levels describe a set of $\sigma + 2\pi$ bonds. So a triple bond description is quite appropriate.

**Acknowledgment.** M.J.C. is grateful to the Calouste Gulbenkian Foundation for its support of her stay at Cornell. M.J.C. also thanks Marja Zonnevylle and Jing Li for sharing their expertise and for helpful discussions. Our work was supported by the National Science Foundation, Grant DMR 821722. We thank Jane Jorgensen and Elisabeth Fields for the drawings and Joyce Barrows for the production of this manuscript.

## Appendix

All the calculations were of the extended-Hückel type,[21] with the tight-binding approach.[22] The parameters are collected in Table V.

The geometry of $NaNb_3O_5F$[1] was used for the three-dimensional structure and simpler models taken from it, as described in the text.

The *k*-point sets were chosen according to the geometrical method of Ramirez and Böhm.[28]

**Registry No.** $NaNb_3O_6$, 117067-24-0; $NaNb_3O_5F$, 104848-57-9; $Ca_{0.75}Nb_3O_6$, 109011-02-1; Nb, 7440-03-1.

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# A New Technique To Calculate Low-Energy Conformations of Cyclic Molecules Utilizing the Ellipsoid Algorithm and Molecular Dynamics: Application to 18-Crown-6

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**Abstract:** We present a new approach of generating low-energy conformations of cyclic molecules. It combines a constrained optimization method known as the ellipsoid algorithm for efficient sampling of sterically allowed conformations and molecular dynamics simulations for local exploration of the conformation space around the structures obtained with the ellipsoid algorithm. The methods are applied to the ionophore 18-crown-6. It is shown that this approach can find the conformations obtained in earlier theoretical and experimental investigations, as well as locate new low-energy conformations which have not been previously studied. The energy evaluation of the conformers obtained by the ellipsoid algorithm was carried out using the AMBER molecular mechanics force field. We provide preliminary data which indicate, contrary to current practice, it may not always be appropriate to scale the 1–4 electrostatic interactions when using this force field.

Computational chemistry has profited greatly by the increased size and speed of computers in recent years. Chemists now routinely perform ab initio level calculations[1] on a wide variety of molecules. Larger molecules, including proteins, are being studied by increasingly complex computational methods,[2] including molecular mechanics and dynamics. However, researchers using such methods are hampered by the number of local minima in complex molecules. To avoid the danger of becoming trapped in local minima, one often selects "good" starting structures; this can, however, lead to a biased result. The problem of local minima is especially acute for cyclic molecules since, owing to the interdependence of torsion angles,[3] it is not trivial to obtain an unbiased sampling of closed-ring structures.

Feasible starting structures for energy refinement methods can be generated for acyclic molecules by dihedral grid searches,[3d] but the extension of these searches to cyclic systems is more difficult and such a method quickly expands beyond the realm of practicality as the size of the system increases. Graphics modeling, while rapid, suffers both from bias and the difficulty

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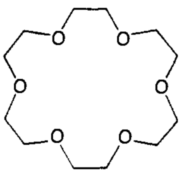
in maintaining ring closure during the modeling process. The techniques of molecular dynamics and Monte Carlo simulation offer partial solutions. They depend on good starting structures, and their extensive use is restricted due to their CPU[4] requirements. Nguyen and Case[5] have recently used a constrained Newton–Raphson method to locate stationary points on the molecular mechanical potential energy surface of cyclooctane and have found this method to be successful, albeit CPU intensive. Various algorithms have been reported that generate structures satisfying given geometric constraints which can be used as starting structures for an energy minimization method such as molecular mechanics. Distance geometry, as presented by Crippen and Havel,[6] attacks the ring-closure problem most elegantly. It is, however, expensive in the sense that the number of degrees of freedom is equal to three times the number of atoms involved. A recently published algorithm[7] reduces the number of degrees of freedom to the number of torsion angles. While no systematic evaluation of the sampling of these two distance geometry methods is published, simple test cases[8] show that neither of them results in an even sampling of the allowed conformation space.

In this paper we describe a new approach to calculate low-energy conformations of cyclic molecules. We have combined the use of a method known as the ellipsoid algorithm[9] with molecular dynamics to generate low-energy conformations of 18-crown-6 (I). The ellipsoid algorithm is a fast and robust method of constrained optimization[10] in that it often finds the global minimum. In the present application, it is used to obtain a large number of structures of compound I that have (1) well-defined bond lengths and bond angles, (2) proper ring closure, and (3) all other interatomic distances larger than the sum of the van der Waals radii of the two atoms involved. The local conformational space around each of these structures is then explored with molecular dynamics. We chose to examine I for our study because its size and flexibility posed a nontrivial challenge for a conformational search. Also, experimental investigations of I have provided a fairly good understanding of the conformers which populate the ground state.[11a–d] In addition, an exhaustive search of ideal "diamond lattice" conformations of 18-crown-6 has been made.[11e]



I

**Table I.** Force-Field Parameters for 18-Crown-6 (I)

| bond | $K_r$ (kcal/Å/mol) | $r_{eq}$ | |
|---|---|---|---|
| C–C | 260.0 | 1.526 | |
| C–H | 340.0 | 1.098 | |
| C–O | 320.0 | 1.425 | |
| angle | $K_\theta$ (kcal/radian) | $\theta_{eq}$ | |
| C–O–C | 100.0 | 111.8 | |
| C–C–H | 35.0 | 109.5 | |
| C–C–O | 80.0 | 109.5 | |
| H–C–H | 35.0 | 109.5 | |
| H–C–O | 35.0 | 109.5 | |
| dihedral[a] | $V_n$ (kcal/mol) | $\gamma$ | $n$ |
| X–C–O–X | 1.15 | 0.0 | 3 |
| X–C–C–X | 1.30 | 0.0 | 3 |
| O–C–C–O | 0.14 | 0.0 | 3 |
| O–C–C–O | 0.50 | 0.0 | 2 |
| C–C–O–C | 0.38 | 0.0 | 3 |
| C–C–O–C | 0.20 | 180.0 | 2 |
| VDW[b] | $R^*$ | $\epsilon^*$ | |
| C | 1.80 | 0.06 | |
| H | 1.54 | 0.01 | |
| O | 1.65 | 0.15 | |
| electrostatic | | $q$ | |
| C | | 0.244 | |
| H | | -0.021 | |
| O | | -0.406 | |

[a] X stands for any atom type. [b] The factors $A$ and $B$ of the 6-12 potential are defined as $A = \epsilon^* R^{*12}$ and $B = 2\epsilon^* R^{*6}$.

## Methods and Procedures

Initially the molecular ring of I was cut open by breaking one of the carbon–carbon bonds. The ellipsoid algorithm then re-formed this bond by optimization with respect to constraints on selected atom–atom distances. The degrees of freedom hereby were the torsion angles about single bonds. Therefore, condition 1 (see above) had to be checked only for the starting structure. Conditions 2 and 3 were imposed by upper and lower bounds on interatomic distances based on the AMBER force field.[12] For the broken bond, the equilibrium carbon–carbon bond length was used. In addition, proper bond angles around this bond were imposed by using the 1–3 distances defined by the force field equilibrium angle. For all these distances, lower and upper bounds were chosen that identify a permissible range of ±5% of the ideal distance. Lower bounds were defined for all atom pairs whose distance was a function of at least one dihedral angle in order to prevent collapsing of the molecule. They corresponded to the distance for which the Lennard-Jones potential of the AMBER force field resulted in an energy of 1 kcal/mol.

Every conformation of the linear molecule (with one broken bond) corresponded to a point in $\mathbf{R}^{n-1}$, where $n$ is the number of bonds along the molecular ring, i.e., the number of torsion angles; there is no dihedral angle defined for the broken bond. Each calculation was started by *randomly* choosing a point in $\mathbf{R}^{n-1}$, representing a starting conformation. An ellipsoid with radius $(n - 1)^{1/2} \cdot 180°$ was then defined around this point; it contained the entire conformational space of the molecule.[13] The ellipsoid algorithm then proceeded by generating a sequence of ellipsoids with constantly decreasing volumes, each of which should contain at least one solution. The centers of these ellipsoids were the conformations obtained at every iteration. Each iteration was based on the gradient of either a single violated upper distance limit or of the sum of violations of all the van der Waals constraints of the current conformation. Details of the implementation of the algorithm can be found in earlier publications where the ellipsoid algorithm was used for the determination of polypeptide structures[13] and for docking two possibly flexible molecules.[14]

The structures obtained with the use of the ellipsoid algorithm were refined with molecular mechanics (MM) using the AMBER[12] force field. The point charges for I were derived from an electrostatic potential calculation.[15] The force-field equation is defined in eq 1 and the force field parameters[16] are listed in Table I. An all-atom approach was used;

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(4) Abbreviations: CPU, Central Processing Unit; FPS, Floating Point Systems; LEMAO-3G, Least Energy Minimal Atomic Orbitals at the 3-Gaussian level; MD, Molecular Dynamics; MM, Molecular Mechanics; RMSD, Root Mean Square Difference; STO-3G, Slater-Type Orbitals (3 Gaussian functions).

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$$E_{\text{total}} = \sum_{\text{bonds}} \frac{K_r}{2}(r - r_{\text{eq}})^2 + \sum_{\text{angles}} \frac{K_\theta}{2}(\theta - \theta_{\text{eq}})^2 + \sum_{\text{dihedrals}} \sum_{n} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right] + \sum_{\substack{i<j \\ \text{1-4 terms}}} \left\{\frac{1}{2}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right]\right\} + \sum_{\text{H bonds}}\left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right] + \sum_{i<j}\left[\frac{q_i q_j}{\epsilon R_{ij}}\right] + \sum_{\substack{i<j \\ \text{1-4 terms}}} \left\{\frac{1}{\text{EEL}_{\text{scale}}}\left[\frac{q_i q_j}{\epsilon R_{ij}}\right]\right\} \quad (1)$$

that is, all methylene hydrogens were explicitly represented.[16b] Each degree of freedom was relaxed during the minimizations hydrogens I a constant dielectric ($\epsilon = 1$) was used. The molecules were minimized until the root-mean-square gradient was less than 0.001 kcal/Å. Normal mode analysis was used to check that each of the conformers of I described explicitly in this text represented local minima and not saddle points on the conformational potential surface. Some structures were further examined with constant-temperature molecular dynamics (MD) simulations using the AMBER software package[17] and based on the same force field as for the MM calculations. These simulations were run using the SHAKE[18] option for carbon-hydrogen bonds; they lasted 10 ps each and at the end of every 1 ps the resulting structure was saved and subsequently quenched by MM minimization.

Ab initio single-point calculations were made on selected conformers of I. We used the program Gaussian 80-UCSF[19] for these calculations, utilizing both the STO-3G[20] basis set and the LEMAO-3G (least energy minimal atomic orbitals at the 3-Gaussian level)[21] basis set.

## Results

The ellipsoid algorithm was used to generate 200 conformations of I. Of these runs, 65 showed no remaining violations of the geometric constraints defining ring closure and the van der Waals radii of each atom; 85 had violations of less than 0.05 Å. One run did not converge at all and was excluded from further examination. The average time/run was 12 CPU min on a VAX 11/750 running under the 4.3 BSD UNIX operating system; the 65 converging runs took on the average less than 6 CPU min. The ellipsoid optimizations were stopped if convergence was not met after 3000 iterations; at this point the ellipsoid had become so small that it described essentially a single conformation.[13] The average number of iterations for the 65 converging runs was 1040. Each of the ring dihedral angles in the generated conformations of I was classified as gauche$^+$ (g$^+$), gauche$^-$ (g$^-$), or anti (a),[22] and an examination of the structures showed each to be unique on the basis of this description.

The initial energies of the conformers were evaluated using the AMBER force field (see eq 1) with the normal 1-4 electrostatic energy scaling factor: EEL = 0.5 (Figure 1A). Two subsets of energies can clearly be seen in Figure 1A. The high-energy structures (≥140 kcal/mol) correspond to those conformers which showed at least one violation greater than 0.2 Å at the end of the ellipsoid runs. The 199 conformers were then minimized using the same force field, and the results of these calculations are shown in Figure 1B. Each conformer took, on average, 12 CPU s to converge during the MM minimization using a FPS-264 running under the 1.0 SJE operating system. A scatter plot of the initial ellipsoid conformer energies versus the energy minimized structures is shown in Figure 2. There is little correlation between the two energies, and thus one cannot preselect only the most favorable structures for subsequent energy minimization using the criterion of initial conformer energy.

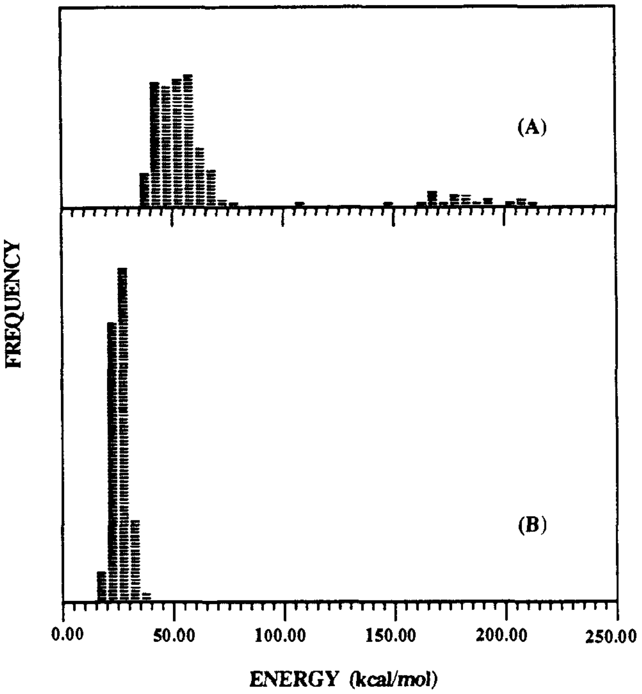

**Figure 1.** Histograms of 18-crown-6 conformer energies before (A) and after (B) molecular mechanical minimization using the AMBER force field. The structures were energy minimized employing an EEL scale factor of 0.5 for the 1-4 electrostatic contribution (see eq 1). Histogram (A) shows the 197 structures, out of 200 structures, which were in the range of the energy scale, and (B) contains the energies for 199 conformers. One run of the ellipsoid algorithm did not converge (see text).

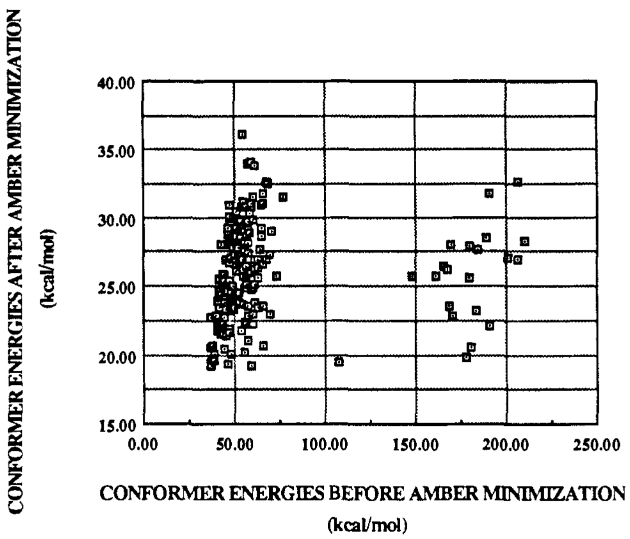

**Figure 2.** Scatter plot of 18-crown-6 conformer energies evaluated with the AMBER force field after ellipsoid optimization versus the energies evaluated subsequent to refinement using AMBER. The structures were minimized using an EEL scale factor of 0.5 for the 1-4 electrostatic energy; 197 of the 200 conformers are depicted.

Previous experimental[11a-d] and theoretical[11e,16a,23,24] investigations have indicated that the lowest energy conformers of uncomplexed I are the $C_i$ structure Ia and the $D_{3d}$ structure Ib (Figure 3 and Table II). Neither of these conformers was found after the energy minimizations of the ellipsoid structures. However, this is not surprising since convergence under the ellipsoid algorithm requires only that specified distance constraints are not violated and the

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**Table II.** Classification of Conformers Ia–Ik in Terms of Dihedral Angle Ranges

| compd | COCC1 | OCCO1 | CCOC1 | COCC2 | OCCO2 | CCOC2 | COCC3 | OCCO3 | CCOC3 |
|---|---|---|---|---|---|---|---|---|---|
| Ia | g+ | g− | a | a | g+ | a | a | a | a |
| Ib | a | g+ | a | a | g− | a | a | g+ | a |
| Ic | g+ | g+ | a | g+ | g− | a | a | g+ | a |
| Id | g+ | g+ | a | g+ | g− | a | a | g− | a |
| Ie | g+ | g+ | a | g− | g+ | a | a | g− | a |
| If | g+ | g+ | a | a | a | a | g− | g+ | a |
| Ig | g+ | g+ | a | a | g− | a | a | g+ | g− |
| Ih | g+ | g− | a | g− | g+ | a | g+ | g− | a |
| Ii | g+ | a | a | g+ | g− | a | a | a | a |
| Ij | g+ | g+ | a | a | a | a | g− | g+ | a |
| Ik | g+ | a | a | a | g+ | a | a | g− | a |

| compd | COCC4 | OCCO4 | CCOC4 | COCC5 | OCCO5 | CCOC5 | COCC6 | OCCO6 | CCOC6 |
|---|---|---|---|---|---|---|---|---|---|
| Ia | g− | g+ | a | a | g− | a | a | a | a |
| Ib | a | g− | a | a | g+ | a | a | g− | a |
| Ic | a | g+ | a | a | g− | a | a | g− | g+ |
| Id | g− | g+ | a | a | g− | a | a | g− | g+ |
| Ie | a | g+ | a | a | g− | a | a | g− | g+ |
| If | a | g− | a | a | g− | a | g− | g+ | a |
| Ig | a | g+ | g+ | g+ | g− | a | a | g+ | a |
| Ih | g− | g+ | a | g+ | g− | a | g− | g+ | a |
| Ii | g+ | g− | a | a | g+ | a | a | g+ | a |
| Ij | a | g− | a | a | g− | a | g− | g+ | a |
| Ik | a | g+ | g− | a | a | a | a | g− | a |

van der Waals spheres of the atoms do not penetrate each other, whereas the AMBER force field uses a more sophisticated energy evaluation. Since the potential surface described by the AMBER force field contains more minima than does that described by the ellipsoid algorithm, structures became trapped in local minima during energy minimization. We decided to employ MD to search the local regions of conformational space.

Four different conditions were used for the MD simulations: the seven lowest energy refined conformers of I and seven randomly choosen conformers were subjected to constant-temperature MD simulations at 300 and 350 K. Each 10-ps MD simulation required 2.5 CPU min on the FPS-264 running under the 1.0 SJE operating system. The surprising results of these simulations are summarized in Figure 4, A and B. Again, conformers Ia and Ib were not identified among the minimized structures, yet five conformers (Ic–Ig) with AMBER energies lower than both Ia and Ib were found on at least one occasion after quenching the MD coordinates of the runs starting with the seven lowest energy refined structures (Figure 3 and Table II). A decomposition of the AMBER energies of these five conformers showed they shared only one common feature: all five conformers had very favorable 1–4 electrostatic contributions to their total energy.

The ellipsoid algorithm generated structures were minimized again; this time with no 1–4 electrostatic scaling (i.e., EEL = 1.0). Conformers Ia and Ib were not among the resulting minimized structures and, as before, there was minimal correlation between the energies of the initial and MM minimized structures. The dissimilarities of the potential surfaces of I associated with the two electrostatic scaling factors may be appreciated by comparing the MM minimized conformer energies. For surfaces with similar topology, one would expect a correlation coefficient near 1.00 between the two calculated energies of the various conformers. Instead, the correlation coefficient is 0.76 which indicates that significant differences exist between the two potential surfaces.

MD calculations were performed as before, except no 1–4 electrostatic scaling was implemented, on the seven lowest energy and seven randomly chosen structures. This time, no conformers were found with lower energies than Ia (Figure 4, C and D). Most pleasing to us was the fact that the lowest energy structure found was Ia, and this conformer was identified during six quenches from two of the runs starting with the seven lowest energy structures. The $D_{3d}$ conformer of Ib was not found during the MD simulations, but four structures (Ih–Ik) were found on at least one occasion with energies between Ia and Ib (Figure 3 and Tables II and III). Two of these structures, Ih and Ii, were generated from MD runs starting from the seven lowest energy MM conformers, while the remaining two structures, Ij and Ik, originated from MD runs using the randomly chosen MM minimized conformers as starting coordinates.

**Table III.** Ab Initio and Molecular Mechanical Relative Energies for Molecular Dynamics Generated Conformers

| | MM (kcal/mol) | | |
|---|---|---|---|
| conformer[a] | EEL = 0.5 | EEL = 1.0 | STO-3G[b] |
| Ia | 0.00 | 0.00 | 0.00 |
| Ib | 0.13 | 1.92 | −0.48 |
| Ic | −0.04 | 5.26 | n.c. |
| Id | −0.35 | 4.01 | 1.25 |
| Ie | −0.84 | 3.95 | 2.74 |
| If | −0.40 | 2.50 | n.c. |
| Ig | −1.16 | 3.91 | 3.39 |
| Ih | −1.05 | 1.27 | −0.50 |
| Ii | 1.90 | 1.46 | n.c. |
| Ij | 0.36 | 1.73 | n.c. |
| Ik | 2.21 | 1.74 | n.c. |

[a] See text for the definitions of Ia–Ik. [b] n.c., not computed.

In an effort to establish what the appropriate choice for the 1–4 electrostatic scaling factor should be, we performed single-point ab initio calculations at the STO-3G[20] and LEMAO-3G[21] basis set levels using MM minimized structures. The LEMAO-3G basis set proved to grossly overestimate the charges on both carbon and hydrogen atoms; consequently the energy differences between the conformers of I varied wildly, and they are not reported in detail. The STO-3G basis set is known[1] to generally underestimate the dipole moment and charge separation in molecules; however, we felt this minimal basis set would provide some gauge of the relative energies of the 18-crown-6 conformers. We calculated the dipole moment at the STO-3G level for the experimental geometry of dimethyl ether[25] and found the calculated value to be 1.28 D, which is only 0.03 D less than the experimental[25] value. Thus, one would expect this basis set to provide an adequate representation of the charge distribution in 18-crown-6. We have compared the results of the STO-3G calculations and MM calculations, using the two scaling factors, in Table III. The STO-3G level calculations place the energies of conformers Id, Ie, and Ig at a higher level than Ia and Ib and the energy of conformer Ih at approximately the same level.

## Discussion and Conclusions

We have presented a new approach for conformational sampling and applied it to 18-crown-6 using two different force-field models,

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(25) Blukis, U.; Kasai, P. H.; Myers, R. J. *J. Chem. Phys.* **1963**, *38*, 2753–2760.

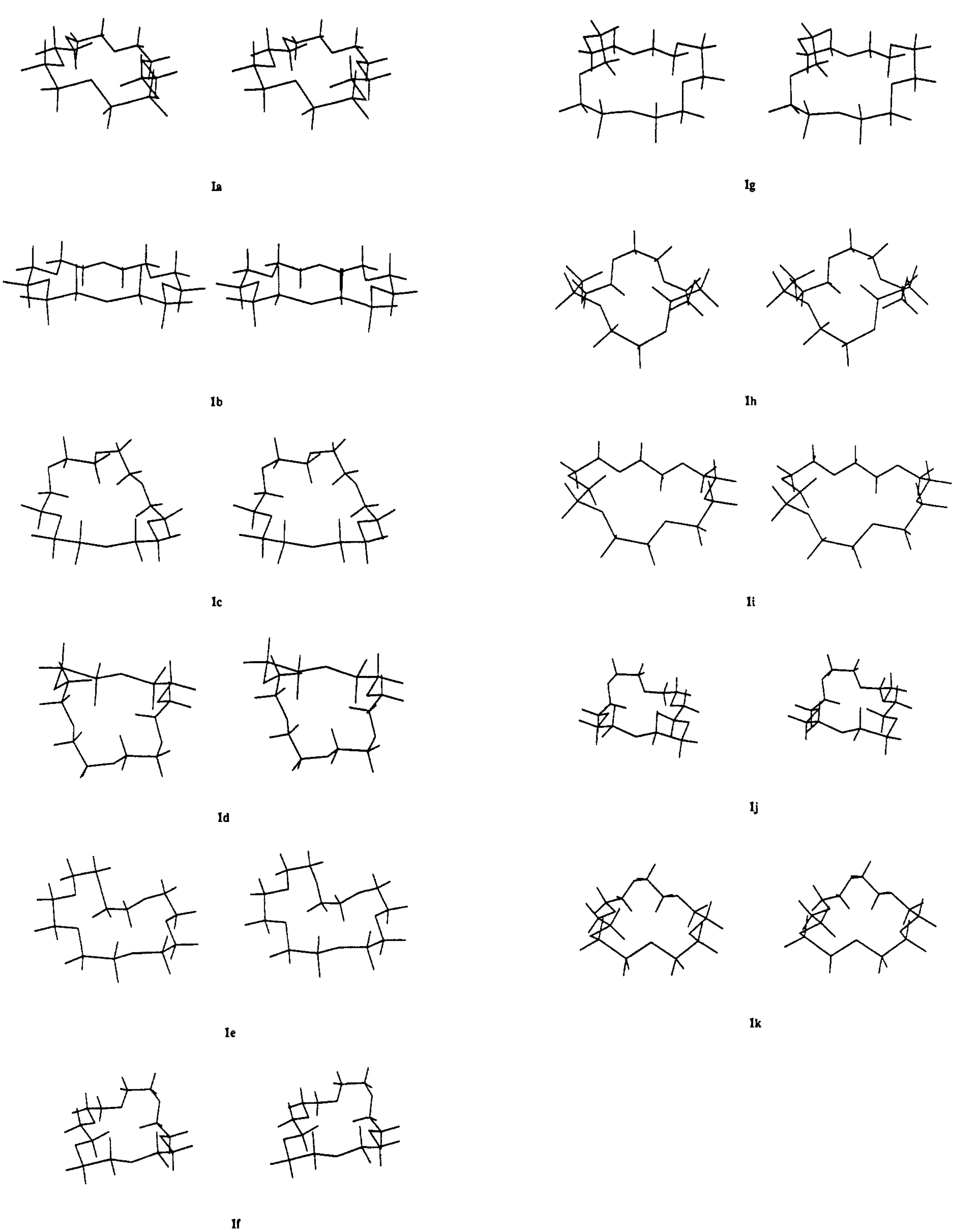

**Figure 3.** Stereoviews of the 18-crown-6 conformers Ia–Ik (see text and Table II).

one with an electrostatic scale factor of 0.5 for atoms separated by three bonds and one without such a scale factor. In order to evaluate the intrinsic difficulty in sampling the low-energy minima and locating the global minimum, the following considerations give an approximate lower bound for the total number of local minima in the AMBER potential surface for compound I. By computing the RMSD (root-mean-square difference) of dihedral angles for 10 structures that were quenched from a single MD run (see Results), an average distance of not more than ≈60° between neighboring local minima around the starting structure is obtained. If the assumption is made that the separation of local minima is approximately the same throughout the entire conformation space, i.e., also in regions of high-energy conformations, the expected total number of local minima is of the order of $6^{12}$ or $10^9$. Here we use 360/60 = 6 structures per degree of freedom and 12 degrees of freedom, i.e., 18 torsion angles minus 6 ring-closure constraints.

Since the well-known "$C_i$" conformation (Ia) is the observed conformation in the crystal,[11] it is not unreasonable that it might be the global minimum in the gas phase also, given that there are no very strong intermolecular interactions between individual molecules in that crystal. Our method either located this conformation or found structures with lower energies with respect to the force field used. Several structures were found with energies within a range of 2 kcal/mol from the lowest energy structure. Conformer Ib, which has an energy 1.92 kcal/mol higher than

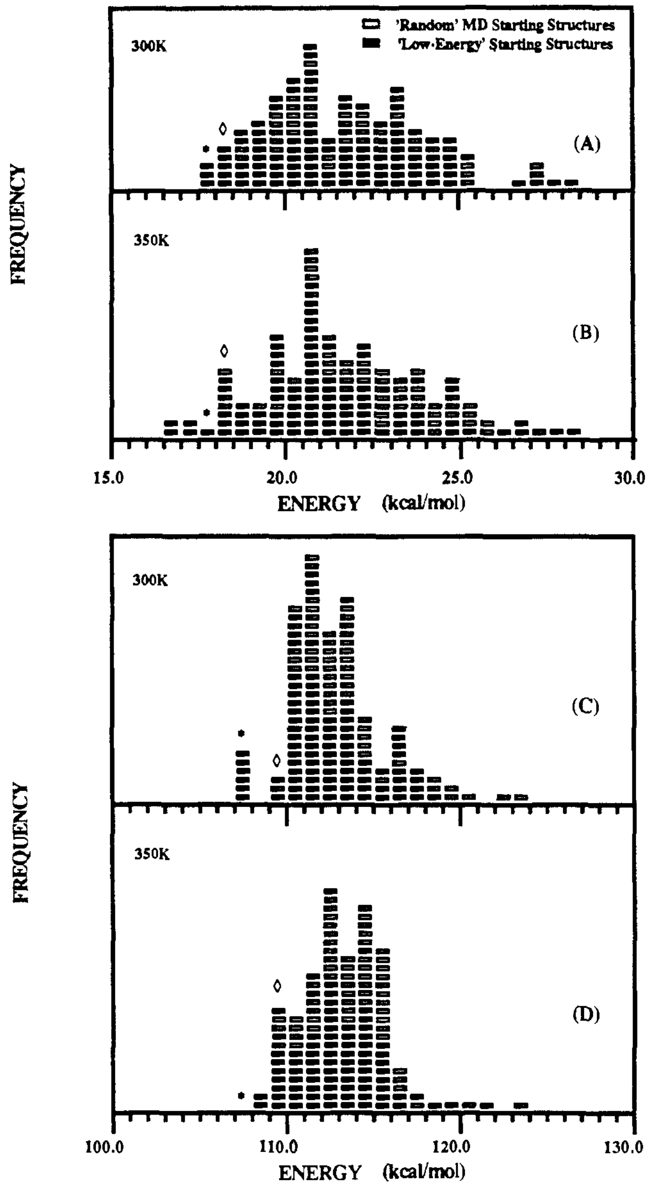

**Figure 4.** Histograms of 18-crown-6 conformer energies after molecular dynamics and energy refinement using the AMBER force field. The specific conditions during MD and MM were ~ respectively temperature during MD, 1-4 electrostatic scaling factor; (A) 300 K, EEL = 0.5; (B) 350 K, EEL = 0.5; (C) 300 K, EEL = 1.0; (D) 350 K, EEL = 1.0. For reference, the molecular mechanical energies of Ia and Ib have been indicated by the symbols * and ◊, respectively. The significance of the terminology "low-energy" and "random" is discussed in the text.

the (presumable) global minimum Ia (Table III), was, however, not located. It has statistically a small chance to be found by an algorithm with good sampling characteristics. To show this we make an analogy to the Boltzmann distribution. Consider a two-state system where the states are separated by 2 kcal/mol. According to Boltzmann statistics, the low-energy state would be found 29 times as often as the high-energy state. The chances of locating the latter one decrease further as more states of intermediate energies are introduced.

As has been shown here and in earlier publications,[13,14] many runs of the ellipsoid algorithm converge to zero error long before the ellipsoid had collapsed to essentially one point. These runs also took less than about half the average time of all runs. It seems therefore advisable to stop the ellipsoid program early, rejecting runs that do not converge, and to spend the time on a new run. In principle, the ellipsoid algorithm might be expected to have problems with ring closure.[13] For cyclic molecules, six degrees of freedom must be subtracted from the number of torsion angles. Therefore, the feasible region is part of an $n - 6$ dimensional subspace and thus has a zero volume in $\mathbf{R}^n$. Numerical problems might arise when the ellipsoid algorithm tries to approximate this region. This problem can be avoided by choosing finite ranges for the ring-closure constraints. The application described in this paper shows that even for ranges as small as ±5% no numerical problems are encountered.

The ellipsoid part of the combined method presented here takes less CPU time than the molecular mechanics/molecular dynamics calculations. Experience with AMBER and other programs indicates that 1 FPS-264 CPU min equals approximately 100 VAX 11/750 CPU min. One thus would like to screen the intermediate results for promising candidates and only examine these with molecular mechanics and dynamics. Figure 2 indicates, however, that an elimination just after the ellipsoid optimization is not advisable. A close look at RMSD values between the 199 structures obtained from the ellipsoid program showed no correlation with the results from the energy minimization. Although we did not attempt an exhaustive investigation of the effects of temperature and initial energies of the starting structures during the MD simulations, several observations may be made from our data. We have calculated the average and the standard deviation of the energies obtained for the runs at different temperatures and from different initial energy ranges of the starting conformations. Each statistic is based on 70 structures, that is, 7 conformers were each subjected to 10 ps of MD with the resulting coordinates saved after every 1 ps. For both electrostatic scaling factors the differences between the average energy reached after MD is smaller than the standard deviation. Figure 4, however, seems to indicate that preference should be given to low-energy structures for the molecular dynamics runs. On the other hand, in the calculations based on an 1-4 electrostatic scale factor of 0.5, conformer Ih was missed. Its energy after minimization was in the lower half of all 199 energy values; however, it was not among the seven lowest that were selected for further MD processing. The final energy of this conformer was the second best of all the structures obtained, regardless of the value of the electrostatic scaling factor (Table III). Since the energy width for the various groups in Table III does not scale by the same factor as the 1-4 electrostatics, we can state that the energy surface for compound I is not dominated by that energy contribution.

The question of which molecular mechanical electrostatic scale factor is correct for the gas phase is a complex one. Weiner et al.[12] introduced the use of both 1-4 van der Waals and electrostatic scale factors in their development of the united-atom force field for proteins and nucleic acids. The 1-4 van der Waals scaling made physical sense, since a 6-12 function overestimates short-range repulsion and electronic polarization effects would tend to reduce 1-4 exchange repulsion effects. But the 1-4 electrostatic scale factor of 0.5 was included because it allowed the united-atom model to more closely mimic the results of all-atom simulations than without the scale factor. In the all-atom paper,[16b] it was demonstrated in a number of test cases that using a 1-4 van der Waals scale factor of 0.5 or 1.0 gave similar conformational profiles, but no careful examination of what scale factor to use for 1-4 electrostatic energies was done. Quantum mechanical calculations[1] might give an answer to this based on gas-phase energies. The STO-3G basis set is not accurate enough to give a definitive answer, although our results seem to suggest that the most appropriate choice for the 1-4 electrostatic scaling factor for the AMBER force field is 1.0 (Table III). Both the 3-21G and 4-31G split valence basis sets tend to overestimate charge separation and dipole moments; thus these basis sets are also deficient and consequently they were not used. Although the 6-31G* basis set would provide more accurate one-electron properties, the size of the calculations is prohibited given our computer resources. Occam's razor (when in doubt, use the simplest model) also supports a scale factor of 1.0, i.e., no "special" treatment of the 1-4 electrostatic effects. Further studies on peptides and other model systems are being carried out to analyze this issue more fully.

The conformations generated using the ellipsoid algorithm are typically more compact than those obtained using the AMBER force field. The respective average radii of gyration are 3.1 ± 0.1 Å for the ellipsoid structures and 3.3 ± 0.1 Å after MM minimization and quenched MD. Both the MM and MD radii of gyration averages were calculated for those conformers obtained with a 1–4 electrostatic scaling factor of 1.0. The X-ray structures of the $C_i$ and $D_{3d}$ conformers of I are yet more expanded with radii of gyration of 3.4 Å. These differences in the radii of gyration may be largely accounted for by two factors. In the ellipsoid algorithm optimization, electrostatic forces are neglected during the generation of molecular conformations, whereas the AMBER force field evaluates the electrostatic forces as pairwise monopole–molopole interactions (eq 1). Furthermore, lower bounds were used to describe the VDW repulsive forces in the ellipsoid algorithm rather than a smooth potential as was used in the MM force field. An increase in the lower bounds might more closely mimic the AMBER force field, but this would have to be done judiciously since it would also diminish the number of solutions. A decomposition of the conformer energies supports the hypothesis that both factors account for the differences in the radii of gyration, but their relative importance appears to be dependent on the particular conformation.

Most of the conformers generated by the ellipsoid algorithm and subsequent MD simulations were of $C_1$ symmetry although we did find conformers of the $D_{3d}$, $S_2$, $C_3$, $C_2$, and $C_i$ point groups. Perhaps the most interesting conformer which was generated was Ih. This conformer, like Ib, is of the point group $D_{3d}$. It is one of the four structures with energies intermediate to that of Ia and Ib which were generated during MD simulations using a 1–4 electrostatic scaling factor of 1.0; in the case of an electrostatic scale factor of 0.5, this structure has the second best energy. With respect to the ab initio calculations it has the lowest energy of all the structures Ia–Ik. The most striking feature of this structure is that is possesses six pseudo-corners[11c] [$g^{\pm}$, $g^{\pm}$, a (where the gauche dihedrals are of opposite sign)]. This is in contrast to Ib, which has no pseudo-corners but rather possesses the low-energy dihedral sequence [a, $g^+$, a]. Consequently, Ib enjoys a very favorable dihedral energy contribution to its total energy. It does so, however, at the expense of a favorable electrostatic interaction since the oxygens dipoles are all oriented toward the center of the ring. Conformer Ih compensates for its higher dihedral energy by providing a very advantageous arrangement of the oxygen atoms. The oxygen atoms lie in two planes and each oxygen is staggered with respect to the oxygen atoms in the alternate plane.

The $D_{3d}$ conformation Ib, which is the observed conformation in the crystal[11] for an 18-crown-6 adduct with $K^+$, $Rb^+$, or $Cs^+$, is not found in either set of quenched dynamics runs, even though it is only ca. 2 kcal/mol less stable than the lowest energy uncomplexed structure with either electrostatic model. In our earlier study,[24a] we found that putting a $K^+$ into a $C_i$ conformation of 18-crown-6 caused it to isomerize to $D_{3d}$ even during molecular mechanics minimization. In the general case, one would like to select conformations that will bind another molecule. To study this, we have taken our three lowest energy structures from MD with EEL = 1.0 (Ia, Ih, and Ii), placed a $K^+$ ion at the center of mass, and then run 10 ps of quenched dynamics on these structures. We find the $D_{3d}$ conformation Id two times among these structures and the $K^+/D_{3d}$ structure was the lowest energy complex among the $K^+$/18-crown-6 conformers found. Thus, here, as in our earlier study,[24a] we can rationalize the observed crystal structure. Furthermore, we can imagine the general use of ligands such as $K^+$ to "select" conformations of "receptors" that are capable of interacting favorably with such ligands.

Whereas other methods may rely on good starting structures (e.g., MM and MD), or have been applied to 18-crown-6 or similarly complex systems only with additional constraints (e.g., distance geometry[23]), our approach starts from completely random initial conformations and yet uses no additional requirements other than the force-field parameters. The combined method presented here may thus well be the method of choice for conformational sampling of systems with a complexity of the order of 18-crown-6. On the other hand, as shown by Uiterwijk et al.,[11e] a complete search of idealized diamond lattice conformations of 18-crown-6 can be done. Our approach is clearly less "exhaustive" than theirs, but lends itself more easily to slightly larger and less symmetric systems, where exhaustive searching becomes prohibitive. In addition, by definition, the diamond lattice method will find only those conformers in which the internal coordinates are ideal (e.g., in the case of 18-crown-6,[11e] the allowed dihedral angles are 60, 180, and 300°). Of the 11 conformers shown in Table II, only six were found using the diamond lattice method.[11e] However, one could imagine combining the diamond lattice method to generate initial structures with the molecular mechanics/dynamics part of the search strategy presented herein. This might be the method of choice for analyzing the conformations of some molecules.

For large systems, the use of additional constraints on specific distances or torsion angles during the implementation of the ellipsoid algorithm becomes necessary. These constraints can define ligand binding[14] or be based on NMR experiments.[13,26] For the latter case, calculations with the ellipsoid algorithm have been completed which show that it can successfully handle up to 188 degrees of freedom.[27] Future applications will treat complexed systems using additional degrees of freedom for docking a cation (e.g., $K^+$) to a flexible 18-crown-6 and/or will include the complete AMBER energy function during the ellipsoid optimization except for the bond length and bond angle potentials which are not varied when the degrees of freedom are defined by the torsion angles.

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