JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. **12**, 858–864 (1999)

Stochastic conformational search of brefeldin A and experimental structures

Mark Froimowitz[1]*, Douglas J. Gordon[1], Adel Moussa[1], Reem M. Haidar[1] and Clifford George[2]

[1]Pharm-Eco Laboratories, Lexington, MA 02421, USA
[2]Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375-5341, USA

Received 12 May 1999; revised 21 July 1999; accepted 27 July 1999

epoc

ABSTRACT: The macrocyclic lactone brefeldin A was used as a test molecule for the MM3-92 Stochastic Search conformational searching algorithm. A number of low energy conformers were found and 12 of these were also subjected to *ab initio* energy minimization using the 3–21*G** and 6–31*G** basis sets with the Spartan 5.0 package. There was reasonable agreement between the MM3-92 and *ab initio* results in that almost all of the conformers found by the former were also stable low energy conformers with the latter. The structure of breflate, an ester analog of brefeldin, was also determined by X-ray crystallography. There was very good agreement between experimental structures for the compound and the lowest energy conformer determined by the *ab initio* methods. This was also among the lowest conformers found by MM3-92. Despite some imperfections in the MM3-92 force field, the Stochastic Search method was a powerful method for determining molecular conformation. Copyright © 1999 John Wiley & Sons, Ltd.

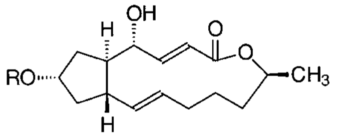
KEYWORDS: *ab initio*; brefeldin A; breflate; crystallography; conformation; energy minimization; MM3-92; stochastic search

Additional material for this paper is available from the epoc website at http://www.wiley.com/epoc

INTRODUCTION

Performing a conformational search in a molecular modeling study can be a difficult task for all but the smallest molecules due to large numbers of possible conformers. For example, there are 3^{10} possible conformers in a molecule with 10 bonds about which rotation can occur, where each dihedral angle can have one *trans* and two gauche values. To be sure that the lowest energy minimum (the global minimum) has indeed been found, it may be necessary for all of these conformers to be evaluated. Conformational searching in a cyclic compound presents another problem in that the set of dihedral angles that describe the ring must allow ring closure. A number of methods have been developed to allow the efficient determination of the global minimum of moderately sized molecules. One promising method, which is available as a subroutine in the MM3-92 program, is Stochastic Search, which utilizes a Monte Carlo approach of generating random crude structures which are then energy minimized.[1–3] There are some recent reports of the use of the Stochastic Search method for the conformational analysis of crown ethers.[4,5] We wanted to test the Stochastic Search method and brefeldin A appeared to be an ideal test case because it contains a medium size ring and also because the molecule, while moderately complex, does not contain any exotic atom types.

Brefeldin A is a macrocyclic lactone natural product with a wide range of antibiotic activities.[6] Because of these biological properties, as well as others, there has been a strong interest in its total synthesis,[7–12] including a stereoselective synthesis of the biologically active enantiomer.[13] A crystal structure of brefeldin has been determined and used to assign the absolute configuration.[14] A previous molecular modeling study of brefeldin and some of its derivatives, which only reported a limited number of torsion angles, found that a rigid and planar lactone ring was necessary for its cytotoxic and antifungal activities.[15]



brefeldin: R = H
breflate: R = $N(CH_3)_2CH_2CO$

In addition to the MM3-92 energy minimizations, *ab initio* quantum mechanical calculations using different basis sets were performed on the lowest energy conformers found by MM3-92. These included energy minimizations using the 3–21*G** basis set, 6–31*G** single point calculations on the 3–12*G** structures, and energy

---

Correspondence to: M. Froimowitz, Pharm-Eco Laboratories, 128 Spring Street, Lexington, MA 02421, USA.
E-mail: markf@pharmeco.com

Copyright © 1999 John Wiley & Sons, Ltd. CCC 0894–3230/99/110858–07 $17.50

minimizations using the 6–31G^* basis set. This allows a detailed comparison of different methods for determining conformational energies and geometries and provides insight into the reliability of these methods. Also, a novel crystal structure was obtained for breflate, an ester prodrug of brefeldin that has better solubility in water.[16] Finally, a detailed conformational analysis was performed on brefeldin by NMR spectroscopy.[17]

Experimental

MM3-92

The MM3-92 program[18] and parameter set was used for the molecular mechanics studies of brefeldin. The only missing parameters for the compound were the torsional parameters for the sequence $H–C_{sp2}–C_{carbonyl}–O_{sp3}$. As computed by 6–31$G^*$ *ab initio* calculations on $H_2C{=}CH{—}COOCH_3$, the energy difference between the two conformers in which the carbonyl is *trans* or eclipsed to the double bond is 0.6 kcal/mol in favor of the latter. This was used to set the torsional parameters to $V1 = 1.2$, $V2 = 10.0$, and $V3 = 0.0$. After the initial conformational searches, it was clear that there were some problems with the computed structure of the allyl alcohol moiety in brefeldin. However, the 1996 parameter set for MM3 had improved values of the torsional constants for $C_{sp2}–C_{sp2}–C_{carbonyl}–O_{sp3}$ ($V1 = -0.5$, $V2 = 1.0$ and $V3 = -0.985$) and these were used to compute the final reported MM3-92 structures.

Conformational searching was performed with the Stochastic Search feature of the MM3-92 program to generate possible conformations of brefeldin. Briefly, this method randomly moves the atoms in a starting conformation a certain distance depending on a specified parameter and a generated random number. The resulting crude structure is then energy minimized in two stages. The first minimization is with the block diagonal method since this is a more robust and faster method for a crude structure with a very high energy. After this, the full matrix method, which produces more consistent final structures, is used to achieve the final minimized conformation. The newly generated conformer must then meet certain criteria, such as having the correct stereochemistry, to be accepted. If these criteria are met, the conformer is stored and the process is then repeated to generate a new conformer. Typically, low energy conformers tend to be found many times within a run. The Stochastic Search feature is particularly suited for dealing with medium sized ring structures, such as in brefeldin, since systematic conformational searches are more difficult due to the necessity of finding sets of dihedral angles that close the ring.

There are several parameters that must be set for the Stochastic Search method. PUSHORIG controls the maximum distance that any atom is allowed to move. For the first two runs, this was set at 0.50 Å. However, the molecule tended to return repeatedly to the previous conformation with this value of the parameter. For later runs, therefore, a value of 0.75 Å was used and the molecule was much less likely to return to the previous conformation. A second parameter is FRAN which, if it is less than a random number (0 to 1), causes the program to obtain the next starting structure from the previous one. This was set to 0.01 so that this was the most likely occurrence. Otherwise, the starting structure was chosen from conformations that had been previously generated. The parameter WIDTH, which was set to 0, causes the next structure to be randomly selected from the previous stored conformation rather than by an exponential weighting function. The parameter ADDIN is an energy increment that is added to a previously stored conformer each time it is selected as a starting conformer in order to prevent the same one from being chosen repeatedly and this was set to 0.5.

In this work, each independent run was set to contain 2000 energy minimized conformers that were generated from a single starting conformation. In all, 12 such runs were performed, each with a different starting conformation. The starting conformations were varied to try to ensure that the conformation space was fully explored. The first few starting conformations were low energy conformers, whereas the later ones were high energy conformers found in previous runs and some effort was made to choose conformers with quite different dihedral angles.

Since brefeldin contains five chiral centers, it was necessary to use the feature that checks for the correct stereochemistry. That is, the crude structure that is initially generated must have all of the same chiral centers. After the energy minimizations, the stereochemistry is again checked and a conformer is only stored if it has preserved the correct stereochemistry. Brefeldin also contain two *trans* double bonds. The version of the program that we used, however, does not check for the correct configuration about double bonds, and conformers with *cis* double bonds were eliminated later.

A number of changes were made to the MM3-92 source code to correct possible errors* and to reduce the CPU time necessary for the execution of the code. First, the original program performed both a block diagonal and a full matrix minimization of the starting conformation. This is problematic for compounds with chiral centers since the full matrix minimization causes the program to lose track of the starting stereochemistry and can invert it to its mirror image.[19] This was fixed by only performing a block diagonal minimization for the starting structure. However, there was still a problem of chiral inversion with the full matrix minimizations that are the last step for each new conformer. Therefore, if the first chiral center had

---

*One of the reviewers indicated that the problems with chiral inversions and energy going up have been fixed in the 1996 and later versions of MM3.

Copyright © 1999 John Wiley & Sons, Ltd.

been modified during the course of the minimization, the conformer was automatically inverted to its mirror image. The test for the correct stereochemistry was then carried out once again to ensure that it had not changed.

A second change that was made to the source code concerns the number of energy minimizations that are carried out for each new crude conformer that is generated. Since the starting crude conformer typically has quite a high energy, the initial block diagonal minimization would often stop at another high energy form because the minimization would terminate, most often due to the computed energy going up after a particular step. However, it was found that repeated cycles of block diagonal minimizations would usually result in conformers with much lower energies. Therefore, five cycles of block diagonal minimizations were performed for each new crude conformation prior to the full matrix minimization. Also, the stereochemical inversions that occur during the energy minimizations almost always did so during these initial block diagonal minimizations since they were performed on structures with very high energies. Since the full matrix minimizations are considerably more expensive in terms of the required CPU time, it was advantageous to check for the correct stereochemistry prior to the full matrix minimization as well as after. If the stereochemistry had changed after the block diagonal method, the conformer was discarded at that point. These changes resulted in a much higher percentage of lower energy forms being generated.

After a conformer has been energy minimized, the program computes its moments of inertia and tests to see if these and the computed energy match a previously generated conformer. If they do not, this is a new conformer, which is then stored. If they do, the conformer has previously been generated and the counter for that conformer is incremented. Thus, one obtains a file which contains all unique conformers and how often they were found. After the MM3-92 program has been run, another program, named SELECT, which is also part of the MM3-92 package, sorts the accepted conformations by their energies and tabulates how frequently each one has been found. This program was modified to exclude structures that had negative vibrational frequencies (i.e. transition states) and which contained *cis* double bonds, since brefeldin only contains *trans* double bonds. The number of *cis* double bonds was relatively small. After this, the various individual Stochastic Search runs were merged into a single file and the SELECT program was rerun. The final output contained all of the local minima found sorted by energy and this makes it easy to see if the same conformer has been found repeatedly in different runs.

Ab initio methods

The 12 lowest energy MM3-92 conformers of the 13-membered ring in brefeldin were transferred to the Spartan Version 5.0 molecular modeling package.[20] Initial *ab initio* calculations were performed with the 3–21*G** basis set with full energy and geometry optimization. The 3–21*G** geometries were also subjected to a single point calculation using the 6–31*G** basis set. Finally, full energy and geometrical optimization of the 3–21*G** structures was performed with the 6–31*G** basis set.

Superposition studies

Least squares superpositions of various conformers of brefeldin obtained by various computational and experimental methods were performed with the PCMODEL program.[21]

Single crystal X-ray analysis of breflate

A clear plate 0.16 × 0.18 × 0.32 mm crystal of breflate $C_{20}H_{31}NO_5$ was selected for data collection. Data was collected on a computer controlled diffractometer with an incident beam graphite monochromator (Bruker P4 with Cu Kα radiation, $\lambda = 1.54178$ Å, $T = 295$ K). A least-squares refinement using 35 centered reflections within $19 < 2\theta < 55°$ gave the monoclinic P2$_1$ cell, $a = 6.043(2)$, $b = 11.826(4)$, $c = 15.012(4)$ Å, $\beta = 96.94(2)°$ with $V = 1064.9(6)$ Å^3 $Z = 2$, and $d_{\mathrm{calc}} = 1.14$ g/cm^3. A total of 2348 reflections were measured in the $\theta/2\theta$ mode to $2\theta_{\max} = 115°$, of which there were 1721 independent reflections. Corrections were applied for Lorentz and polarization effects. A face indexed numerical absorption correction was applied, $\mu = 0.66$ mm^{-1}, and maximum and minimum transmission were 0.91 and 0.80, respectively. The structure was solved by direct methods and refined on F^2 with a full matrix least-squares SHELXTL97.[22] The 238 parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms bonded to oxygen were refined while those bonded to carbon utilized a riding model in which coordinate shifts of carbons were applied to the attached hydrogens with bond lengths of 0.96 Å, idealized bond angles, and U_{iso} (H) set to either 1.2 U_{eq} (C) or 1.5 U_{eq} (C) for methyl groups. The final R values for all the independent data were $R1 = 0.053$, and $wR2 = 0.128$. The goodness of fit parameter was 1.12 and final difference Fourier excursions were 0.13 and −0.15 eÅ^{-3}, Tables of atomic coordinates, bond distances, bond angles, anisotropic thermal parameters and additional data collection and refinement parameters have been submitted to the Cambridge Crystallographic Data Centre and are available as supplementary material from the epoc website at http://www.wiley.com/epoc.

Atom numbering

To facilitate comparisons, the same numbering conven-

Copyright © 1999 John Wiley & Sons, Ltd.

Table 1. Dihedral angles (°) and steric energies (kcal/mol) of low energy MM3-92 conformers of brefeldin classified by different conformers of the 13-membered ring

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
13-membered ring												
C9–C5–C4–C3	56	57	64	71	62	74	66	70	59	54	50	61
C5–C4–C3–C2	−113	87	−111	−107	73	91	87	−109	93	61	87	−110
C4–C3–C2–C1	179	179	179	177	−176	180	−179	174	180	−177	−176	178
C3–C2–C1–O	0	−1	−174	−175	2	0	−2	−175	−3	175	2	0
C2–C1–O–C15	−144	145	135	138	143	140	136	137	140	−140	158	−139
C1–O–C15–C14	59	−106	−76	−60	−151	−79	−90	−120	−93	48	−145	67
O–C15–C14–C13	70	75	89	−63	42	−66	−70	46	84	63	94	77
C15–C14–C13–C12	−146	−148	−150	149	62	149	154	64	−144	−153	−51	−146
C14–C13–C12–C11	74	73	68	−94	−146	−77	−89	−150	77	72	−61	84
C13–C12–C11–C10	91	90	98	114	119	−100	98	128	−115	95	162	−112
C12–C11–C10–C9	180	179	176	172	−171	−179	176	−171	−175	180	179	−176
C11–C10–C9–C5	124	126	131	132	131	−30	121	141	−22	117	133	−29
C10–C9–C5–C4	−88	−85	−78	−73	−69	−72	−72	−67	−78	−103	−77	−78
Five-membered ring												
C6–C7–C8–C9	39	38	29	19	18	15	21	18	28	44	28	28
C7–C8–C9–C5	−42	−43	−42	−39	−39	−36	−40	−39	−42	−37	−42	−42
C8–C9–C5–C6	28	31	39	43	44	44	43	45	39	15	40	40
C9–C5–C6–C7	−4	−7	−22	−32	−33	−35	−31	−34	−23	12	−23	−23
C5–C6–C7–C8	−22	−19	−4	8	9	13	7	10	−3	−34	−3	−3
Hydroxyl groups												
O–C7–C6–C5	−140	−136	−122	−108	−106	−105	−109	−107	−121	−152	−121	−120
O–C7–C8–C9	159	157	147	137	135	133	138	136	146	162	147	147
H–O–C7–C6	−71	−71	−73	−73	−75	−73	−74	−73	−73	−73	−71	−72
O–C4–C5–C9	175	177	−178	−171	−179	−167	−174	−173	179	173	169	179
O–C4–C3–C2	128	−31	131	136	−44	−28	−32	133	−25	−58	−30	132
H–O–C4–C5	180	168	−176	−177	175	163	167	−177	163	180	171	180
Steric energy	33.0	33.4	32.6	33.2	34.6	34.0	34.3	33.8	34.7	35.0	35.3	35.4

tion has been used as in the previously published crystal structure of brefeldin.[14]

RESULTS AND DISCUSSION

MM3-92 Stochastic Search

As can be expected for a 13-membered ring,[23] a considerable number of low energy conformations were found by the Stochastic Search method. In addition to different possible conformers of the 13-membered ring, there are potentially nine different combinations possible for the two hydroxyl groups. Table 1 lists the best 12 conformations of the 13-membered ring, all with the same optimal conformation of the hydroxyl groups. As can be seen, these 12 conformers are within 2.8 kcal/mol of the global minimum. [The ordering of the low energy conformers is based on their energies as computed with the original force field. However, the changes in the force field (see Experimental section) resulted in some reordering of the minima.]

A question that should arise from the Stochastic Search method is how well the conformation space has been sampled and whether the global minimum has been found. To answer this, it should be noted that the lowest energy forms of the 13-membered ring are found in all eight of the independent runs. Thus, it is unlikely that there is a lower energy global minimum that has not been found. However, the method was less successful in picking out different conformations of the hydroxyl groups since the lowest energy combination was found much less frequently than the second lowest. In addition, the lowest energy combination of the hydroxyl groups was not found in every independent run.

Upon visual inspection, conformers I–III and X–XII had the hydroxyl group equatorial on the five-membered ring while the remaining conformers appeared to be intermediate forms. That is, most of the O–C7–C6–C5 dihedral values were in the vicinity of −110° and most of the O–C7–C8–C9 dihedral angles in the vicinity of +140° rather than gauche or *trans* values. This appears to be a flaw in the MM3-92 force field since the *ab initio* results starting from these intermediate MM3-92 structures always produced axial conformers (see below). Similarly, starting a MM3-92 energy minimization from a 6–31G^* geometry with an axial hydroxyl group also produced the intermediate form, suggesting that the 6–31G^* geometry is not stable with the MM3-92 force field.

Ab initio calculations

The 12 lowest energy-minimized structures of the 13-

Table 2. Relative energies (kcal/mol) of *ab initio* 3–21*G** and 6–31*G** results for the lowest 12 conformers of the 13-membered ring found by MM3-92 Stochastic Search

Conformer	3–21*G** energy minimization	6–31*G** single point calculation	6–31*G** energy minimization
I	3.9	3.6	3.7
II	0.0	0.0	0.0
III	3.3	2.4	2.1
IV	3.5	3.8	2.9
V	3.1	4.9	4.3
VI	3.6	5.0	4.6
VII	0.7	2.6	1.9
VIII	5.8	5.4	4.9
IX	2.7	3.1	2.9
X	3.4	4.9	4.8
XI	3.5	3.1	3.1
XII	9.1	7.6	7.9

membered ring found by MM3-92 were also subjected to *ab initio* 3–21G* energy minimization, a single point 6–31*G** calculation for the 3–21*G** geometry, and 6–31*G** energy minimization. These results are shown in Table 2 and the dihedral angles that described the energy minimized structures are listed in the supplementary material and Table 3. First, all of the energy minima found by MM3-92 also appeared to be minima by the *ab initio* energy minimizations methods. However, the ordering of the minima was different. The most notable difference was that the *ab initio* methods found conformer II (Fig. 1) to be the global minimum by a considerable amount as compared with most of the other conformers. For the 6–31*G** calculations, this global minimum was favored by 1.9–7.9 kcal/mol, while it ranged from 0.7 to 9.1 for the 3–21*G** calculations. This compares with a total of only 2.8 kcal/mol range for the MM3-92 results.

Superposition comparisons were also made between the energy minimized structures obtained by the MM3-92 method and the 3–21*G** and 6–31*G** *ab initio* methods. Using all of the atoms in the molecule, the best agreement for the corresponding conformers was between the 3–21*G** and 6–31*G** results with a root mean square (rms) deviation of 0.150 Å. The MM3-92 structural results were actually more consistent with the 6–31*G** results with an rms deviation of 0.384 Å as compared with 0.462 Å for the 3–21*G** results. The major structural differences between the corresponding conformers obtained by MM3-92 and the *ab initio* results involved the cyclopentane ring with an axial alcohol group in the *ab initio* calculations and an intermediate form in the MM3-92 calculations. Given the flexibility of a five-membered ring, it is not surprising that it is the source of most of the structural differences.

The reason for the computed favorability of the global minimum is not entirely clear. For example, *ab initio* calculations with the 6–31*G** basis set for allyl alcohol indicated that the conformation in which the hydroxyl

Table 3. Dihedral angles (°) of 6–31 *G** geometry optimized structures of the 12 lowest energy conformers found by MM3-92

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
13-membered ring												
C9–C5–C4–C3	55	55	62	71	59	71	66	71	60	47	49	57
C5–C4–C3–C2	−117	111	−106	−106	92	108	110	−112	111	91	100	−110
C4–C3–C2–C1	168	−174	167	167	−167	−170	−170	164	−171	−168	−171	165
C3–C2–C1–O	16	1	−145	−151	−15	−8	−6	−153	0	149	0	17
C2–C1–O–C15	−159	165	166	158	160	154	150	154	163	−155	173	−156
C1–O–C15–C14	68	−139	−106	−80	−158	−97	−114	−142	−127	59	−157	71
O–C15–C14–C13	67	66	69	−65	37	−65	−70	40	72	61	84	76
C15–C14–C13–C12	−138	−135	−124	131	61	138	143	61	−130	−150	−53	−137
C14–C13–C12–C11	72	68	70	−86	−143	−77	−89	−139	79	73	−57	83
C13–C12–C11–C10	102	102	116	132	122	−105	114	132	−125	100	166	−119
C12–C11–C10–C9	−176	−178	179	173	−168	178	179	−176	−178	−176	−175	179
C11–C10–C9–C5	120	122	128	133	130	−10	123	140	−5	117	128	−8
C10–C9–C5–C4	−96	−96	−95	−91	−71	−84	−90	−70	−91	−104	−83	−92
Five-membered ring												
C6–C7–C8–C9	43	42	42	−27	−4	−22	−27	−1	−28	42	40	42
C7–C8–C9–C5	−36	−37	−37	3	−22	−4	3	−25	4	−32	−41	−38
C8–C9–C5–C6	16	17	17	22	40	27	22	42	21	9	27	19
C9–C5–C6–C7	10	9	8	−39	−43	−42	−40	−43	−39	17	−3	7
C5–C6–C7–C8	−32	−32	−31	41	30	40	41	28	41	−37	−23	−31
Hydroxyl groups												
O–C7–C6–C5	−152	−152	−151	−74	−86	−76	−73	−88	−74	−156	−143	−151
O–C7–C8–C9	165	165	165	91	115	96	91	118	90	165	163	165
H–O–C7–C6	−65	−65	−64	−62	−64	−62	−62	−65	−62	−65	−66	−65
O–C4–C5–C9	175	179	−178	−170	−179	−166	−170	−169	−176	170	172	178
O–C4–C3–C2	124	−10	136	136	−27	−12	−10	130	−9	−28	−19	132
H–O–C4–C5	177	174	179	−179	180	176	176	−179	175	178	177	177

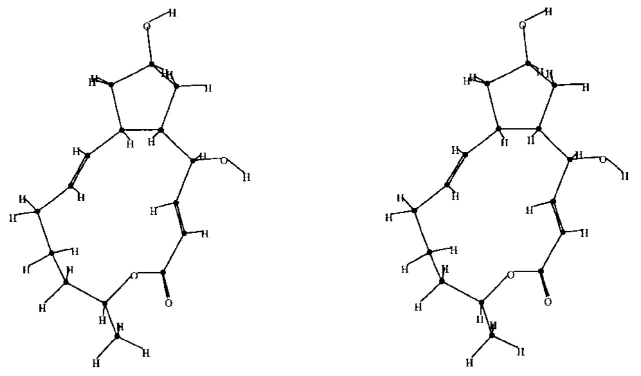


Figure 1. Stereoscopic image of global minimum conformer II as calculated by *ab initio* 6–31G^* calculations

oxygen eclipses the double bond has about the same energy as the gauche conformation. Similarly, of the two stable conformations due to rotation of the bond between the double bond and the ester group, the less favored one (by 0.6 kcal/mol), in which the carbonyl oxygen is *trans* to the double bond, is the one that appears in the crystal structures and in the *ab initio* global minimum. These results also occur with *ab initio* calculations on larger fragments of these sequences (not shown). Thus, the favorability of conformer II appears to be a consequence of the interactions within the whole molecule of brefeldin. One possibility is that the two double bonds, which are across from each other in the ring, are making a highly favorable interaction. The closer of the two double bond hydrogens is 2.9 Å from both carbon atoms of the other double bond. This hypothesis was tested by performing single point 6–31G^* *ab initio* calculations on pairs of ethylene molecules which were fixed in the relative orientations of the 12 6–31G^* minimized structures. The relative energies of the different ethylene dimer orientations varied up to a considerable 13.9 kcal/mol. The relative orientation of the two double bonds in conformer II was the third best one of the 12 and it was favored by 5.3 kcal/mol or better among conformers I, III, IV and V. Thus, the favorability of conformer II may be a consequence of a reasonable conformation of the 13-membered ring which, at the same time, also allows a highly favorable interaction of the two double bonds.

Table 4. Dihedral angles (°) of X-ray crystal structures of brefeldin and breflate derivative

	Brefeldin[a]	Breflate
13-membered ring		
C9–C5–C4–C3	61	58
C5–C4–C3–C2	111	118
C4–C3–C2–C1	−168	−174
C3–C2–C1–O	−3	2
C2–C1–O–C15	170	170
C1–O–C15–C14	−146	−146
O–C15–C14–C13	67	61
C15–C14–C13–C12	−131	−131
C14–C13–C12–C11	70	72
C13–C12–C11–C10	101	107
C12–C11–C10–C9	177	179
C11–C10–C9–C5	118	116
C10–C9–C5–C4	−98	−101
Five-membered ring		
C6–C7–C8–C9	−35	44
C7–C8–C9–C5	12	−34
C8–C9–C5–C6	15	11
C9–C5–C6–C7	−37	15
C5–C6–C7–C8	44	−37
Hydroxyl groups[b]		
O–C7–C6–C5	−71	156
O–C7–C8–C9	83	−166
O–C4–C5–C9	−176	180
O–C4–C3–C2	−8	5

[a] Reference 14.

[b] C7 hydroxyl group is ester in breflate.

X-ray structure

The crystal structure found for breflate is shown in Fig. 2 and the dihedral angles that describe the structure are listed in Table 4. The dihedral angles that describe the

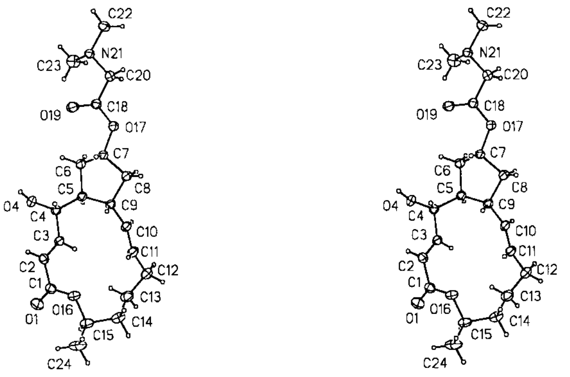


Figure 2. Thermal ellipsoid plot drawn from experimental coordinates of breflate

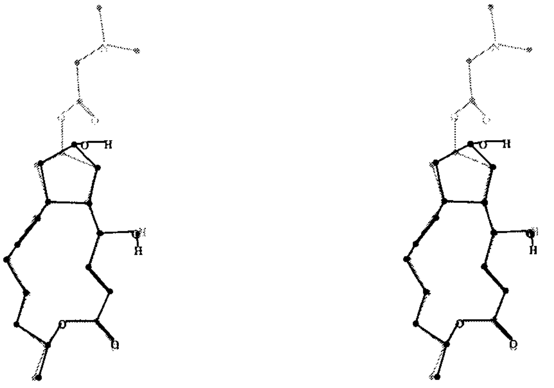


Figure 3. Superposition of crystal structures of breflate and brefeldin

previously published crystal structure of brefeldin[12] are also listed in Table 5. The crystal structures for brefeldin and breflate are superimposed in Fig. 3. As can be seen, the conformations of the 13-membered ring are virtually identical in the two crystal structures. However, there are significant differences between the two structures in the five-membered ring. In brefeldin, the hydroxyl attached to the five-membered ring is axial, whereas it is equatorial in the breflate derivative. However, it is well known that five-membered rings tend to be highly flexible so this difference is not surprising.

In comparing the MM3-92 low energy conformations in Table 1 with the X-ray crystal structures, conformer II, which is 0.8 kcal/mole above the global minimum, is similar to the two crystal structures with respect to the 13-membered ring. Comparing the dihedral angles of MM3-92 conformer II with the crystal structure of brefeldin, six are within 10°, an additional six are within 25°, and one is 40° away from the crystal structure. The global minimum computed by the *ab initio* methods and energetically favored by a significant amount is virtually identical to the structures seen by X-ray crystallography with regard to the 13-membered ring. Comparing the 16 atoms of the two rings of conformer II as computed with the 6–31*G** basis set with the corresponding atoms in the crystal structure of breflate, the rms deviation is only 0.081 Å. The close correspondence of the crystal structures with an *ab initio* global minimum that is favored by a significant amount strongly suggests that there is little structural flexibility in brefeldin. This is also consistent with our recent finding using NMR spectroscopy that brefeldin adopts a very similar conformation of the 13-membered in solution, though the hydroxyl group is axial on the five-membered ring rather than equatorial.[17]

CONCLUSIONS

The MM3-92 stochastic conformational search produced a large number of low energy conformers for brefeldin. When the structures of the lowest 12 were energy minimized by *ab initio* 3–21*G** and 6–31*G** calculations, similar geometrical structures were found to be stable minima. However, as noted above, there are some differences, most notably for axial conformers of the hydroxyl on the five-membered ring as computed by the *ab initio* methods which were intermediate forms by the MM3-92 calculations. Conformers in which the hydroxyl group is axial on the five-membered ring did not appear to be stable structures with the MM3-92 program.

The Stochastic Search method in MM3-92 was found to be a highly robust method for the determination of the molecular conformations of brefeldin since the method was able to provide a list of low energy conformers that could then be examined by *ab initio* calculations. There was reasonable agreement between the MM3-92 results and the *ab initio* results in that corresponding conformers were found by both methods to be stable and almost all had low energies. However, there did appear to be some flaws in the energy minimized MM3-92 structures as compared with the *ab initio* results. The results obtained here suggest that the combination of the Stochastic Search method to determine possible low energy forms and the *ab initio* methods to provide highly reliable structures is a powerful method for the determination of molecular structure.

The very good agreement between the crystal structures of brefeldin and breflate and the NMR structure of brefeldin in solution with regard to the conformation of the 13-membered ring suggests that this conformation is intrinsically favored since it predominates in different molecular environments. A very similar conformation that is greatly favored by the *ab initio* calculations strongly bolsters this conclusion.

Acknowledgements

The authors wish to acknowledge useful discussions with Robert Glaser. CG would also like to acknowledge the support of the Office of Naval Research.

REFERENCES

1. M. Saunders, *J. Am. Chem. Soc.* **109**, 3150–3152 (1987).
2. M. Saunders, *J. Computat. Chem.* **10**, 203–208 (1989).
3. M. Saunders, K. N. Houk, Y.-D. Wu, W. C. Still, M. Lipton, G. Chang and W. C. Guida, *J. Am. Chem. Soc.* **112**, 1419–1427 (1990).
4. B. P. Hay, J. R. Rustad, J. P. Zipperer, and D. W. Wester, *J. Mol. Struct. (Theochem)* **337**, 39–47 (1995).
5. M. D. Paulsen, J. R. Rustad, and B. P. Hay, *J. Mol. Struct. (Theochem)* **397**, 1–12 (1997).
6. *The Merck Index*, 12th Edition, Merck, Whitehouse Station, NJ, 1996.
7. E. J. Corey, R. H. Wollenberg, and D. R. Williams, *Tetrahedron Lett.* 2243–2246 (1977).
8. R. Baudouy, P. Crabbé, A. E. Greene, C. Le Drian, and A. F. Orr, *Tetrahedron Lett.* 2973–2976 (1977).
9. P. A. Bartlett and F. R. GreenIII, *J. Am. Chem. Soc.* **100**, 4858–4865 (1978).
10. A. E. Greene, C. Le Drian, and P. Crabbé, *J. Am. Chem. Soc.* **102**, 7583–7584 (1980).
11. Y. Köksal, P. Raddatz, and E. Winterfeldt, *Angew. Chem. Int. Ed. Engl.* **19**, 472–473 (1980).
12. M. Honda, K. Hirata, H. Sueoka, T. Katsuki, and M. Yamaguchi, *Tetrahedron Lett.* 2679–2682 (1981).
13. T. Kitahara, K. Mori, and M. Matsui, *Tetrahedron Lett.* 3021–3024 (1979).
14. H. P. Weber, D. Hauser, and H. P. Sigg, *Helv. Chim. Acta* **54**, 2763–2766 (1971).
15. M. J. C. Crabbe and V. Betina, *Folia Microbiol.* **39**, 445–448 (1994).
16. L. R. Phillips, T. L. Wolfe, L. Malspeis, and J. G. Supko, *J. Pharm. Biomed. Anal.* **16**, 1301–1309 (1998).
17. R. Glaser, D. Shiftan and M. Froimowitz, *Magn. Reson. Chem.* (in press).
18. Quantum Chemistry Program Exchange, Department of Chemistry, Indiana University, Bloomington, IN.
19. M. Froimowitz, *Quantum Chem. Program Exch. Bull.* **2**, 54 (1983).
20. Spartan, version 5.0. Wavefunction Inc., Irvine, CA.
21. PCMODEL. Serena Software, Bloomington, IN.
22. Sheldrick, G. M., SHELXTL97 program for the refinement of crystal structures. University of Göttingen, Germany.
23. M. Froimowitz, *Biopolymers* **30**, 1011–1025 (1990).

Copyright © 1999 John Wiley & Sons, Ltd.