storage and the isomerization quantum yields seems to leave the model presented in Table II and Figure 11 as the most reasonable model. Of course, this model does not apply to the unlikely possibility that the first step of the vision process does not involve isomerization.

The present analysis of the energy storage in bathorhodopsin provides, for the first time, a *consistent* simulation of storage of a large amount of excess energy. It is shown that a very conservative estimate of the rigidity of the protein simulates trapping the chromophore in a strained conformation that stores a significant amount of energy. It is also shown how a light-induced charge separation can store about 30 kcal/mol if the protonated nitrogen is moved to a nonpolar region of the protein. It is suggested that a large amount of steric energy is likely to be converted into electrostatic energy during the ground-state relaxation of the protein.

The static constraint potential used in this work is only a rough approximation for the ad hoc assumption that the isomerization time is faster than the protein relaxation time. It is clear that a more complete study must involve molecular dynamics simulations of a chromophore in a protein cavity. However, preliminary molecular dynamics studies of the photoisomerization of the chromophore in a cavity of van der Waals molecules have indicated that the protein cavity does restrict the possible isomerization pathways. This point is strongly supported by the fact that the present work could provide a consistent analysis of the relation between the steric constraint of the protein and the quantum yields of formation of bathorhodopsin from rhodopsin and isorhodopsin.

The present model can be considered as a refinement of the previously proposed "bicycle-pedal" model.[6] The bicycle-pedal model simulated the primary event as a cis–trans isomerization around the 11–12 bond accompanied by a rotation around the 15–16 bond (see caption of Figure 2 in ref 6 and Figure 4 of ref 6). Introduction of the protein constraint suggests that the protein cavity prevents a complete rotation around the 15–16 bond due to its resistance to the displacement of the C_{13} methyl. Therefore, the isomerization around the 11–12 bond is accompanied by about 40° twist around both the 9–10 and 15–16 bonds, but the overall pathway is not drastically different from the bicycle-pedal pathway.[32] The key element in both models is that only a concerted twist of two or more bonds can lead to isomerization which will not be blocked by the "viscosity" of the protein cavity at low temperature. The presence of the protein constraints tends to confine the chromophore to concerted twists (along the diagonal of diagrams such as Figure 1) of two angles or more. On the other hand, the intramolecular excited-state potentials (Figure 2) imposes a small barrier for an entirely concerted motion when two angles reach the 90° range at the same time. Thus the isomerization motions should reflect a compromise between the inter- and intramolecular potentials where one angle, e.g., ϕ_{11-12} moves to 90° and another angle, or angles, are twisted by no more than 60°.

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Molecular Orbital Constrained Electron Diffraction Studies. 4. Conformational Analysis of the Methyl Ester of Glycine

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Abstract: The electron diffraction data of the methyl ester of glycine were recorded at 75 °C in order to determine the conformational ground state of the system. The data were analyzed by using geometrical constraints (differences between closely spaced bond distances and bond angles) taken from a previous ab initio MO (4-21G) geometry optimization. On the basis of this analysis it must be concluded that the extended form of the system (I) is the most stable one. The presence of a small concentration (of a few percent) of a second conformer, the semiextended form (II), in the vapors of the compound at 75 °C, can also be inferred from the data. However, because of its dependence on the values of unknown amplitudes of vibration, the existence of measurable amounts of II at 75 °C cannot be established with certainty.

Introduction

The rationale for studies of this kind is based on the contention that, in conformational analyses, it should be advantageous to combine the results of quantum mechanical geometry optimizations with gas electron diffraction data. This is so because the relationship between ab initio calculations and gas electron diffraction is now to some extent complementary. Ab initio calculations can predict rather accurately the primary geometries (bond distances and angles) of simple organic molecules, but they are unreliable in determining small conformational energy differences because of basis set effects, electron correlation, and vibrational effects. Gas electron diffraction (GED), on the other hand, can provide a great deal of conformational information for unperturbed molecules, but this information is often difficult to decipher, because it may be correlated to small differences in primary structural parameters which are not resolved in the data. Assumptions concerning such differences therefore often have to be introduced into GED investigations and can make the results obtained in this way somewhat unreliable.

In molecular orbital constrained electron diffraction (MOCED) studies, those results from quantum mechanical geometry optimization which can be trusted, namely, differences between primary structural parameters of one kind, are used as external constraints of GED data analyses in order to obtain some experimental evidence for the conformational properties of a system of interest. So far the procedure has been applied successfully to the problems of 3-chlorobenzaldehyde,[1] 1,2-dimethylhydrazine,[2]

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[1] N. S. Chiu, J. D. Ewbank, M. Askari, and L. Schäfer, *J. Mol. Struct.*, **54**, 185–195 (1979).

Table I. Estimates of Characteristic Differences of 4-21G Optimized Structures (r_e (ab initio)) Relative to Electron Diffraction r_g Structures[a]

bond distance	$r_g - r_e$(ab initio)
C–H	+0.034(10)(10)
N–H	+0.031(11)(2)
C–C (aliphatic)	−0.008 (3)(2)
C–C(=O)	+0.002(4)(2)
C–O (aliphatic)	−0.023(9)(4)
(O=)C–O	−0.002(9)(4)
C–N (aliphatic)	−0.012(6)(3)
N–C(=O)	+0.020(8)(8)
(O=C)N–C(H_3)	0.000(6)(6)
C=C (nonconjugated)	+0.026(3)(2)
C=O	0.000(4)(4)
bond angles[b]	
X–C–Y	±1.0(20)(10)
C–O–C (aliphatic)	−2.3(10)(2)
C-N–C	−2.5(2)(–)[c]
H–O-C, H-N-C	−4.2(5)(20)

[a] Values for bond distances are in angströms; for bond angles, in degrees. The estimates were taken from a critical evaluation of the 4-21G geometries of more than 30 basic organic compounds by L. Schäfer, C. van Alsenoy, and J. N. Scarsdale, ref 5. The differences $r_g - r_e$ (ab initio) recorded in this table are not weighted averages of cases known, but represent the mean value of the maximum and minimum difference between a reliable r_g structure and a refined 4-21G geometry so far encountered in each category. The deviation of the maximum difference from the mean in each category is given by the number in the second parentheses behind each mean difference recorded; i.e., the numbers in the second parentheses, which refer to the last digits given, define the ranges of $r_g - r_e$ differences so far encountered in different cases. Since the experimental uncertainties of the r_g parameters involved also affect the extensions of $r_g - r_e$ (ab initio) difference ranges, the maximum experimental error of any of the r_g parameters used in this comparison is also listed in each category (numbers in first parentheses). [b] 4-21G bond angles were compared to r_s structures. X and Y symbolize all possible combinations of H, C, N, or O in single and double bonds. When H atoms are involved, the deviations may occasionally extend the range specified by about 0.5°. [c] Only one case available for comparison.

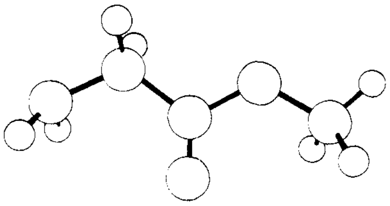

Figure 1. Conformation I of the methyl ester of glycine.

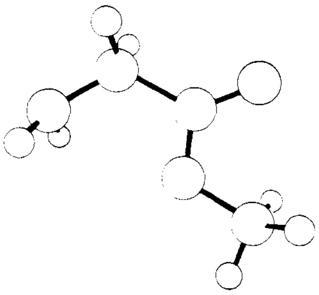

Figure 2. Conformation II of the methyl ester of glycine.

and butene-1.[3] All these cases corroborate the general usefulness of ab initio geometries in interpreting gas-phase structural data[4].

Table II. Some Selected Internal Coordinates[a] and Conformational Energies (*E*, kcal/mol) for Conformations I–IV of the Methyl Ester of Glycine

	I	II	III	IV
C–N	1.458	1.456	1.458	1.462
C–C	1.518	1.526	1.528	1.541
C=O	1.206	1.207	1.201	1.209
C–O(CH_3)	1.355	1.355	1.362	1.342
C–O(C=O)	1.459	1.461	1.450	1.458
N–H	1.002	1.001	1.002	0.998
C–H(CH_2)	1.081	1.082	1.082	1.082
C–H$(CH_3)_{pl}$[b]	1.075	1.076	1.076	1.070
C–H$(CH_3)_{op}$[b]	1.078	1.078	1.080	1.079
C–C–N	113.4	116.4	112.6	119.3
C–C=O	126.1	124.5	122.8	115.8
C–C–O	110.7	112.8	117.5	125.2
O–C=O	123.1	122.7	119.8	119.1
C–O–C	117.9	117.2	123.8	132.0
H–N–C	112.9	112.8	112.4	115.1
H–C–C	108.0	107.0	109.3	104.4
H–C–O_{pl}[b]	105.2	105.1	104.9	111.3
H–C–O_{op}[b]	110.1	110.0	111.3	106.4
C–C–N–H	−63.0	−62.8	−62.2	−113.4
O=C–C–H	−122.4	56.9	−121.5	55.5
H_{op}–C–O–C[b]	−60.4	60.3	61.5	−121.7
E	0.0	1.95	10.70	18.54

[a] All values from the ab initio (4-21G) geometry optimization, ref 8. Bond distances in angströms and angles in degrees. [b] Subscripts pl and op denote in-plane and out-of-plane hydrogens, respectively.

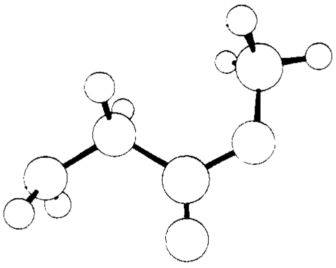

Figure 3. Conformation III of the methyl ester of glycine.

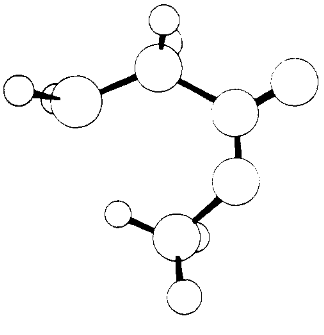

Figure 4. Conformation IV of the methyl ester of glycine.

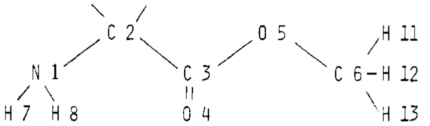

Figure 5. Atom numbering for the methyl ester of glycine.

The contention that the geometries of organic compounds can be predicted reliably by ab initio calculations is still often met with considerable scepticism. In Table I we therefore present the results of a comparison[5] of more than 30 4-21G[6] optimized ge-

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(2) N. S. Chiu, H. L. Sellers, L. Schäfer, and K. Kohata, *J. Am. Chem. Soc.*, **101**, 5883–5889 (1979).

(3) D. Van Hemelrijk, L. Van den Enden, H. J. Geise, H. L. Sellers, and I. Schäfer, *J. Am. Chem. Soc.*, **102** 2189–2195 (1980).

(4) (a) L. Schäfer, H. L. Sellers, F. J. Lovas, and R. D. Suenram, *J. Am. Chem. Soc.*, **102**, 6566–6568 (1980); (b) H. L. Sellers and L. Schäfer, *ibid.*, **100**, 7728–7729 (1978); (c) C. Van Alsenoy, J. N. Scarsdale, and L. Schäfer, *J. Chem. Phys.*, **74**, 6278–6284 (1981).

Table III. Parameter Shifts Obtained by Refinement of the Electron Diffraction Data[a]

	(I)
$\Delta r_1 (N_1-C_2; C_2-C_3; C_3-O_5; C_6-O_5)$	-0.010_8 (0.007)
$\Delta r_2 (C_3=O_4)$	-0.001_4 (0.003)
$\Delta r_3 (N_1-H_7; N_1-H_8; C_2-H_9; C_2-H_{10}; C_6-H_{11}; C_6-H_{12}; C_6-H_{13})$	$+0.033_1$ (0.022)
$\Delta\alpha_1 (C_3-C_2-N_1; C_2-C_3=O_4; C_2-C_3-O_5; C_3-O_5-C_6)$	−0.30° (1.7)
$\Delta\alpha_2 (C_2-N_1-H_7; C_2-N_1-H_8; C_3-C_2-H_9; C_3-C_2-H_{10}; O_5-C_6-H_{11}; O_5-C_6-H_{12}; O_5-C_6-H_{13})$	−2.04° (13.8)
$\Delta\tau (O_5-C_3-C_2-H_9; O_5-C_3-C_2-H_{10})$	±9.52° (12.0)

[a] The parameters were refined in groups identified in the first column on the left; each member in a particular group was shifted by the same increment in each cycle of the data refinement. The total increments listed (Δr_i for distances, $\Delta\alpha_i$ and $\Delta\tau$ for angles) are the differences $r_g - r_e$ (ab initio), between the theoretical values (Table II) and the final refined parameters of the best fitting model of conformation I (col (I)). Parameters not listed in any of the groups were kept constant during refinement at their calculated values (Table II). Numbers in parentheses are experimental error estimates and are three times the standard deviations of the least-squares procedure.

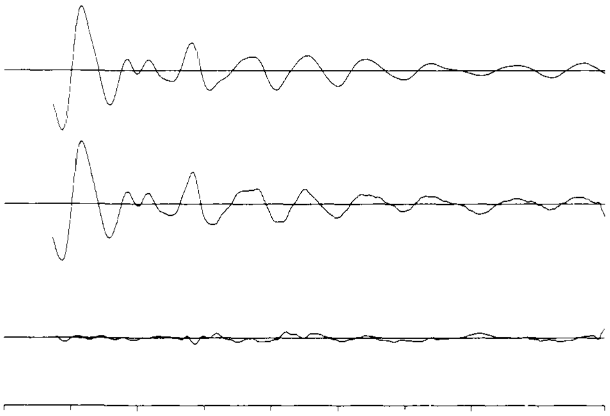

Figure 6. Theoretical (upper curve) and experimental (lower curve) electron diffraction intensities of the methyl ester of glycine from s = 3.625 to s = 45.0 Å^{-1} and the difference between them. See the Experimental Section for details concerning the experiment. The theoretical curve is for conformation I as specified in the Tables III to V. Hash marks on the bottom axis are in units of 5.0 Å^{-1}.

ometries with the corresponding experimental r_g structures. All the geometries used to generate this table were calculated by using Pulay's force method[7] and the 4-21G basis set.[6] The available data (Table I) support the conclusion that 4-21G geometries of many classes of organic compounds are characterized by differences to experimental parameters which are remarkably constant and can be predicted with an accuracy comparable to the usual experimental uncertainty limits.

The case of the methyl ester of glycine (MEG) is ideally suited to test the power of MOCED. A previous 4-21G conformational analysis of the system[8] has yielded four local energy minima (I–IV; see Figures 1–4, Table II, and Figure 5 for the atom numbering). Two of these conformations, III and IV, are so unstable (Table II) that they should clearly not be expected to exist in measurable quantities in MEG vapors at moderate temperatures. The calculated energy difference between the two other forms I and II, however, is so small (less than 2 kcal/mol) that, keeping the approximate nature of the calculations and the size of the system in mind, it is impossible to determine on the basis of the calculations alone which of them is the most stable one. At the same time, a conventional GED study of this system should also be difficult, because the experimental diffraction intensities (Figure 6) yield a radial distribution curve (Figure 7) in which most of the bond distances and the parameters related to bond angles are unresolved.

The MOCED study of MEG described below will show that a clear interpretation of the diffraction data with the help of the ab initio calculations is possible in that conformation I can be identified as the conformational ground state of the system, that measurable concentrations of form II may also exist in the vapors of MEG at 75 °C, but that the data are ambiguous on this point because of uncertainties in mean amplitudes of vibration.

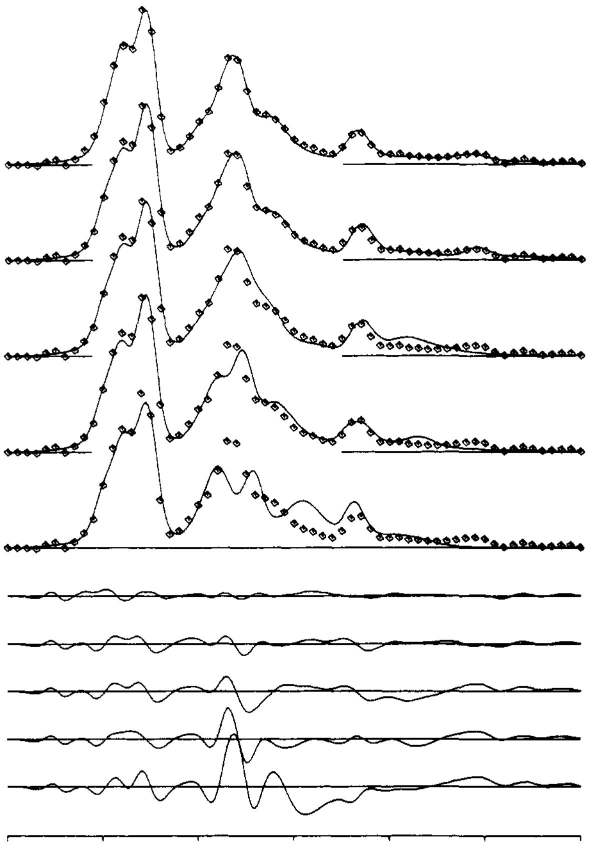

Figure 7. Experimental (dotted lines) and theoretical (solid lines) radial distribution (RD) curves for the methyl ester of glycine. The top curve is for the best fitting refined model of conformation I specified in Tables III to V. The following four RD curves (from top to bottom) are for the unrefined ab initio geometries (Table II) of the conformations I to IV, respectively. The five bottom curves are the differences (experiment minus theory) for the five RD curves described above in the same sequence. Hash marks on the bottom axis are in units of 1.0 Å.

Experimental Procedures and Data Analysis

Fresh samples of glycine methyl ester were prepared before each series of diffraction experiments from commercial samples (Sigma Chemical Co.) of the hydrochloride. Purity of the compound was checked spectroscopically. Diffraction data were recorded photographically at the University of Arkansas electron diffraction unit. Two sets of plates were obtained at 75 °C by using scattering distances of about 10 and 27 cm, yielding curves with s ranges of 10.75–45.0 and 3.625–17.50 Å^{-1}, respectively. The acceleration voltage was approximately 40 keV. The wavelength was calibrated by recording data for C_6H_6 before each experiment and by using the C–C bond length for benzene reported by Bastiansen et al.[9] All photographic plates were evaluated by using our

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(5) L. Schäfer, C. Van Alsenoy, and J. N. Scarsdale, *J. Mol. Struct.*, **86**, 349–364 (1982).

(6) P. Pulay, G. Fogarasi, F. Pang, and J. E. Boggs, *J. Am. Chem. Soc.*, **101**, 2550–2560 (1979).

(7) (a) P. Pulay, Mol. Phys. **17**, 197–204 (1969); (b) *Theor. Chim. Acta*, **50**, 299–310 (1979).

(8) L. Schäfer, C. Van Alsenoy, J. N. Scarsdale, V. J. Klimkowski, and J. D. Ewbank, *J. Comput. Chem.*, **2**, 410–413 (1981).

(9) O. Bastiansen, L. Fernholt, H. M. Seip, H. Kambara, and K. Kuchitsu, *J. Mol. Sturct.*, **18**, 163–168 (1973).

Table IV. Parameter Correlations Obtained by Least-Squares Refinement for a Mixture of I and II[a]

1	1.000						
2	−0.049	1.000					
3	−0.053	0.243	1.000				
4	−0.238	−0.023	−0.014	1.000			
5	−0.043	0.024	−0.072	−0.260	1.000		
6	0.078	−0.060	−0.024	−0.431	0.759	1.000	
7	0.185	0.037	−0.026	−0.105	−0.264	−0.310	1.000

[a] The parameters 1–6 are the distance and angle groups of Table III in the same sequence. Parameter no. 7 represents the composition of the mixture.

digitally filtered microprocessor controlled densitometer.[10] The data were analyzed by applying the usual procedures described before.[11] Our standard tables of electron scattering factors[12] were used. Approximate mean amplitudes of vibration were calculated using our normal coordinate analysis program[13] and the amplitude routine by Stølevik et al.[14] with an order-of-magnitude, ad hoc force field obtained from tables of approximate force constants by Wilson et al.[15]

Experimental and theoretical intensities are shown in Figure 6. Experimental and theoretical radial distribution curves for various models are shown in Figure 7. Results of the parameter refinements are given in Table III. Parameter correlations are given in Table IV. Calculated and data refined mean amplitudes of vibration are given in Table V.

Results and Discussion

At the beginning of this analysis we tested the compatibility of the conformational energy minima of MEG generated by the ab initio study[8] with the electron diffraction data. This was done by comparing the theoretical radial distributions (RD) of the unchanged ab initio geometries[8] (Table II) with the experimental RD curve. This comparison is documented in Figure 7. We note that, even without any refinement, the ab initio radial distribution of I is very close to the experiment, whereas all other models (II to IV) show significant discrepancies. Particularly consider the maximum of experimental radial distribution around 4.9 Å which can be assigned to the $N_1\cdots C_6$ distance in I but to no internuclear distance in the other forms. Also consider the insufficiency of experimental radial distribution to accomodate the same distance in the other models where it is located around or below 4.2 Å. We conclude that form I represents a significantly populated ensemble of MEG in its vapors at 75 °C.

In further analysis the geometrical parameters of the four models I to IV were allowed to vary to adjust theoretical intensities to experimental ones. Variable parameters were collected into six groups described in Table III. The members of a particular group were shifted by the same increment in each cycle of the refinement. Hence, there were six variable geometrical parameters. Constant parameters were constrained to their ab initio values (Table II).

The ab initio values (Table II) were also used as the initial values of the variable parameters. This mode of refinement thus imposed the ab initio differences between the parameters in one group as constraints on the least-squares data refinement. Inspection of Table I reveals that the parameter shifts relative to r_g to be expected for the 4-21G geometries of MEG on the basis of the previous studies are approximately within 0.01 to 0.02 Å and 1–2° (or better) of the bond distance and bond angle shifts, respectively (Δr_i, $\Delta\alpha_i$, Table III), actually obtained from our GED data analysis for the best fitting models of MEG. This accuracy should be sufficient for the purposes of this study and supports our contention that the procedure is basically reasonable.

Table V. Calculated and Refined Mean Amplitudes of Vibration (Å)[a]

group	type	dist	calcd	ref(I)
<1.32	N_1-H_7	1.035	0.072	*b*
	N_1-H_8	1.035	0.072	*b*
	C_2-H_9	1.115	0.079	*b*
	C_2-H_{10}	1.115	0.079	*b*
	$C_3=O_4$	1.204	0.036	0.033 (0.004)
	C_6-H_{11}	1.109	0.079	*b*
	C_6-H_{12}	1.112	0.079	*b*
	C_6-H_{13}	1.112	0.079	*b*
1.32-1.74	N_1-C_2	1.447	0.047	0.047 (0.004)
	C_2-C_3	1.507	0.049	0.049
	C_3-O_5	1.345	0.046	0.046
	C_6-O_5	1.448	0.046	0.046
1.74-2.60	$N_1\cdots C_3$	2.465	0.078	0.079 (0.009)
	$N_1\cdots H_9$	2.025	0.112	0.112
	$N_1\cdots H_{10}$	2.025	0.112	0.112
	$C_2\cdots O_4$	2.418	0.064	0.065
	$C_2\cdots O_5$	2.343	0.071	0.072
	$C_2\cdots H_7$	2.058	0.107	0.107
	$C_2\cdots H_8$	2.058	0.107	0.107
	$C_3\cdots C_6$	2.388	0.074	0.074
	$C_3\cdots H_9$	2.108	0.114	0.114
	$C_3\cdots H_{10}$	2.108	0.114	0.114
	$O_4\cdots O_5$	2.249	0.061	0.061
	$O_5\cdots H_{11}$	2.015	0.111	0.111
	$O_5\cdots H_{12}$	2.082	0.109	0.109
	$O_5\cdots H_{13}$	2.082	0.109	0.109
2.60-3.04	$N_1\cdots O_4$	2.806	0.125	0.124 (0.026)
	$C_3\cdots H_7$	2.750	0.274	0.273
	$C_3\cdots H_8$	2.750	0.274	0.273
	$C_3\cdots H_{12}$	2.682	0.225	0.224
	$C_3\cdots H_{13}$	2.682	0.225	0.224
	$O_4\cdots C_6$	2.701	0.122	0.121
	$O_4\cdots H_7$	2.870	0.448	0.447
	$O_4\cdots H_8$	2.870	0.448	0.447
	$O_4\cdots H_{12}$	2.745	0.424	0.423
	$O_4\cdots H_{13}$	2.745	0.424	0.423
	$O_5\cdots H_9$	2.641	0.225	0.225
	$O_5\cdots H_{10}$	2.641	0.225	0.225
3.04-3.40	$C_3\cdots H_{11}$	3.246	0.110	*b*
	$O_4\cdots H_9$	3.042	0.170	*b*
	$O_4\cdots H_{10}$	3.042	0.170	*b*
3.40-3.96	$N_1\cdots O_5$	3.629	0.077	0.086 (0.019)
	$C_2\cdots C_6$	3.702	0.076	0.085
	$O_4\cdots H_{11}$	3.756	0.136	0.146
3.96-5.65	$N_1\cdots C_6$	4.850	0.097	0.149 (0.093)
	$N_1\cdots H_{11}$	5.635	0.128	0.179
	$N_1\cdots H_{12}$	5.057	0.319	0.371
	$N_1\cdots H_{13}$	5.057	0.319	0.371
	$C_2\cdots H_{11}$	4.350	0.132	0.183
	$C_2\cdots H_{12}$	4.062	0.253	0.305
	$C_2\cdots H_{13}$	4.062	0.253	0.305
	$O_5\cdots H_7$	3.965	0.239	0.291
	$O_5\cdots H_8$	3.965	0.239	0.291
	$C_6\cdots H_7$	5.061	0.341	0.392
	$C_6\cdots H_8$	5.061	0.341	0.392
	$C_6\cdots H_9$	4.005	0.284	0.335
	$C_6\cdots H_{10}$	4.005	0.284	0.335

[a] The amplitudes were refined in groups. Each member of a group was shifted by the same increment in each cycle of the refinement starting with the calculated values (calcd column) as the initial parameters. The "group" column specifies the upper and lower limits (Å) of contiguous segments of the experimental RD curve. Distances falling into a particular segment constitute one group. The distances and the types are identified in the "dist" and "type" columns, respectively. Column ref(I) gives the amplitudes of vibration (Å) obtained by least-squares refinement of the electron diffraction data of MEG for conformation I. Numbers in parentheses behind the first member of each group are experimental error estimates (three times the standard deviations) for the group amplitudes. [b] Held constant during the refinement, see text.

Mean amplitudes of vibration were also refined in groups identified in Table V. The RD curve was divided into segments

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(10) J. D. Ewbank, P. Bowers, J. Pinegar, and L. Schäfer, *Appl. Spectrosc.*, **35**, 540–543 (1981).

(11) L. Schäfer, *Appl. Spectrosc.*, **30**, 123–150 (1976).

(12) (a) L. Schäfer, A. C. Yates, and R. A. Bonham, *J. Chem. Phys.*, **55**, 3055–3056 (1971); (b) H. L. Sellers, L. Schäfer, and Bonham, *J. Mol. Struct.* **49**, 125–130 (1978).

(13) (a) H. L. Sellers, L. B. Sims, L. Schäfer, and D. E. Lewis, *J. Mol. Struct.*, **41**, 149–151 (1977); (b) *ibid*, *QCPE*, No. 339 (1980).

(14) R. Stølevik, H. M. Seip, and S. J. Cyvin, *Chem. Phys. Lett.*, **15** 263–265 (1972).

(15) E. B. Wilson, J. C. Decius, and P. C. Cross, "Molecular Vibrations", McGraw-Hill, London, 1955.

whose borders coincided with the borders of resolved features of experimental radial distribution. The distances within a segment constituted one group whose amplitudes were shifted by the same increment in each cycle of the refinement. Some amplitudes, connected with relatively weak signals in the scattering function, were kept constant at reasonable values, if they shifted to meaningless minima during refinement. For example, agreement between theory and experiment improved slightly, but not significantly, when all 3.0–3.4 Å group amplitudes were reduced from their calculated values. All amplitudes refined in this way significantly depend on the constraints. Hence, the parameters of Table V are not presented as observables but as the effective parameters of a typical run to characterize the analysis.

In the manner just described, we first tested the ability of the individual models I–IV to reproduce the experimental radial distribution of MEG. We found that only in the case of conformation I parameter variations were able to reduce the differences between theoretical and experimental radial distribution to a level of insignificance (Figure 7). In all other cases (II–IV) there were significant incompatibilities. We conclude that conformation I is the conformational ground state of MEG.

In a second round of data refinements subject to the same constraints described above, we tested the compatibility of conformational equilibria involving pairs of conformations with the experimental data. The combinations I + II, I + III, and I + IV were considered yielding concentrations of up to 3–4% of II in refined mixtures of I and II; 100% of I in the refinement of I and III; and up to 3–4% of IV in refined mixtures of I and IV. It was found that it was not meaningful to test other possible binary combinations without I because none of them was able to reproduce the maximum of experimental radial distribution around 4.9 Å.

The theoretical RD curves of the optimized mixtures of I with II and I with IV are visually practically identical with the one shown for 100% of I in Figure 7. In the latter there are still some minor differences between theory and experiment, probably because of inaccuracies in the chosen constraints. It can be ruled that these discrepancies are insignificant for our conclusions since they are negligibly small and further refinements with different constraints (not following the procedure defined) gave essentially the same results.

In general we like to interpret the results obtained by testing mixtures in the following way. The small concentrations of II and IV obtained in this way are not definitely established by the diffraction data. They depend significantly on the exact magnitudes of the amplitudes of vibration, which are unknown, and they are very uncertain (typical error estimates are in the ±20% range). The mixture of I and IV does not represent a meaningful explanation of the diffraction data because the calculated energy difference between I and IV is too large (18.5 kcal/mol, Table II). This posible interpretation of the diffraction data must therefore be considered an artifact of the refinement. The mixture of I and II cannot be excluded on the same basis because, as stated above, the calculated energy difference between I and II (1.95 kcal/mol) is within the uncertainty limits of the 4-21G approximation. However, because of the relatively small concentration of II, its value obtained from GED data analysis is also very uncertain, and this interpretation of the diffraction data may also be an artifact of the refinement. We emphasize the fact that the error limits include 0% of II and 100% of I.

If the possibility of obtaining a stable minimum for the mixture of I and II in the GED data refinement is taken as an indication for the existence of measurable amounts of II in vapors of MEG at 75 °C, then a concentration of 3% (±20) of II would correspond to a free-energy difference of ΔG_{348} = 2.4 kcal/mol. The lower limit of this value, related to the ±20% concentration uncertainty, would be ΔG_{348} = 0.8 kcal/mol. It is interesting to compare this result for the methyl ester of glycine with the one obtained for the free acid itself; in free glycine the experimental energy difference between the conformations related to I and II was smaller than the ab initio energy difference.[4a] Additional studies have shown[16] that this discrepancy can be rationalized in terms of electron correlation effects.

Conclusion

This study has shown that, in cases of this kind, ab initio calculations may be helpful in interpreting electron diffraction data which in turn may be useful in interpreting small and uncertain conformational energy differences obtained by ab initio calculations.

In the case of the methyl ester of glycine, analysis of the available data—subject to the special constraints defined above and to the general assumptions on which GED data are usually based (see, e.g., ref 11)—leads to the conclusion that conformation I is the conformational ground state of this compound.

The data are also consistent with the existence of a small, measurable concentration (of a few percent) of a second conformation, II, in the vapors of MEG at 75 °C. However, because of uncertainties in mean amplitudes of vibration, the presence of II in measurable amounts is not definitely established by this analysis.

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Registry No. Glycine methyl ester, 616-34-2.

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(16) C. E. Dykstra, R. A. Chiles, and M. D. Garrett, *J. Comput. Chem.*, **2**, 266–272 (1981).