1400 J. Org. Chem. 1993, 58, 1400–1406

Calculation of NMR Observables from Computed Conformers as a Tool of Structure Verification. Confirmation of the Stereochemistry of *trans*-Methyl 2,3-Dimethyl-5-methoxy-2-cyclohexene-1-carboxylate

Jeffrey T. Martin,[†] Per-Ola Norrby,* and Björn Åkermark*

Royal Institute of Technology, Department of Organic Chemistry, S-100 44 Stockholm, Sweden

Received September 15, 1992

Four different methods (MM2, Chem3D, AM1, and MNDO) have been employed to calculate all the low-energy conformers of *trans*- and *cis*-methyl 2,3-dimethyl-5-methoxy-2-cyclohexene-1-carboxylate (1 and 2). For all of these conformers, NOE effects and vicinal coupling constants have been calculated, the latter using two different Karplus-type equations. From these calculations, 12 different sets of NMR observables were computed by taking a population weighted average over all conformations. These results were then used to verify the originally assigned trans configuration of compound 1. The diastereoisomer 2 was also prepared and shown to have the cis configuration. The different methods and the main sources of error are discussed. We found the best method for our purposes to be calculation of NOE from molecular mechanics structures.

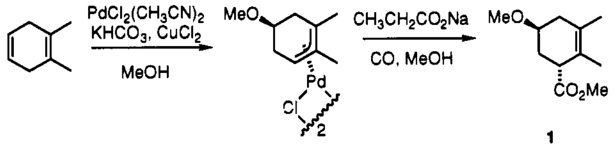
Some time ago, we reported an easy route to functionalized cyclohexene compounds from 1,4-cyclohexadienes, via stoichiometric palladium-promoted addition (Scheme I).[1] Since a wide variety of 1,4-cyclohexadienes are readily available by a number of routes from aryl precursors[2] and since a catalytic conversion has recently been developed,[3] this provides a potential synthesis of difunctionalized cyclohexenes with well defined stereochemistry.

Based on ^{1}H-NMR data and chemical conversion, a trans configuration was assigned to compound 1. It seemed clear, however, that NMR assignments in cyclohexenes may be more difficult than in cyclohexanes because of low barriers to inversion and far lower bias against axial conformations.[4] This is evident from the low value for the axial–axial coupling $J_{41,5}$ (7.1 Hz) in compound 1 which is not substantially different from the axial–equatorial coupling $J_{42,5}$ (5.3 Hz). This moderate difference is also observed in an early study of deuteriated cyclohexene which gave the values 6.77 and 5.67 Hz for the couplings $J_{4,5}$ (our numbering).[5] It is not a priori clear if this is due to contributions from boat-like conformations. Because cyclohexenes are common elements in a wide range of organic compounds, it seemed interesting to investigate the preferred conformation of a disubstituted cyclohexene such as 1. It also seemed interesting to see if it was possible to determine the relative stereochemistries of the isomers 1 and 2 based on calculated NMR data for the two in combination with experimental data for only one of them. Two different kinds of NMR data were used, vicinal coupling constants and NOEs. In order to complete the study, the isomer 2 was prepared by partial epimerization of 1 and isolation of 2, albeit only in ca. 80% isomeric purity, by preparative HPLC.

1 2

Figure 1. The numbering of methylene hydrogens in compounds 1 and 2. H_{41} and H_{61} are cis to the 5-methoxy group.

Scheme I



1

Calculation of Conformations. The calculations were performed with the two molecular mechanics programs Chem3D Plus[6] and MM2(87)[7] and with the semiempirical program Mopac 6.0,[8] using both the MNDO and the AM1 calculation packages. First, a screening of conformations was performed using dihedral drivers in MM2. The conformations obtained were then used as starting structures in all programs. Conformations with an energy more than 3 kcal/mol higher than the optimum conformation were rejected, since these will not contribute significantly to average coupling constants. An additional dihedral driver screening was performed in Mopac, but no additional low energy minima were found.

Calculation of Vicinal Coupling Constants. Two Karplus type equations were used for calculation of vicinal coupling constants. The first and simpler was the classic eq 1, in the modification by Bothner-By.[9] The second was eq 2 developed by Imai and Osawa.[10] This extended equation is explicitly parameterized for population averaging of conformations from a molecular mechanics

---

[†] Present address: Medical Products Agency, Box 26, S-751 03 Uppsala, Sweden.

(1) Söderberg, B. C.; Åkermark, B.; Hall, S. S. *J. Org. Chem.* **1988**, *53*, 2925–2937.

(2) (a) Ryan Zilenovski, J. S.; Hall, S. S. *J. Org. Chem.* **1981**, *46*, 4139–4142. (b) Flisak, J. R. Ph.D. Thesis, The State University of New Jersey, Rutgers, 1985. (c) Birch, A. J.; Subba Rao, G. In *Advances in Organic Chemistry—Methods and Results*; Taylor, E. C., Ed.; Marcel Dekker: New York, 1972; Vol. 8, pp 1–66 and references therein.

(3) (a) Larock, R. C.; Lu, Y.-D.; Bain, A. C.; Russell, C. E. *J. Org. Chem.* **1991**, *56*, 4589–4590. (b) Söderberg, B.; Åkermark, B. Unpublished results.

(4) Corey, E. J.; Feiner, F. *J. Org. Chem.* **1980**, *45*, 765–780.

(5) Auf der Heyde, W.; Lüttke, W. *Chem. Ber.* **1978**, *111*, 2384–2395.

(6) *Chem3D Plus 3.0* for Macintosh, Cambridge Scientific Computing Inc., 875 Massachusetts Avenue, Suite 61, Cambridge, MA 02139.

(7) *MacMimic/MM2(87)* for Macintosh, InStar Software AB, IDEON Research Park, S-233 70 Lund, Sweden.

(8) *Mopac 6.0*, QCPE 455, authored by M. B. Coolidge and J. J. P. Stewart.

(9) Bothner-By, A. A. *Adv. Magn. Reson.* **1965**, *1*, 195.

(10) Imai, K.; Osawa, E. *Magn. Res. Chem.* **1990**, *28*, 668–674.

0022-3263/93/1958-1400$04.00/0

Table I. Observed and Calculated Vicinal Coupling Constants for Compound 1

entry	conf. calc[a]	Karplus eq[b]	amount eq[c] (%)	$J_{1,61}$	$J_{1,62}$	$J_{41,5}$	$J_{42,5}$	$J_{5,61}$	$J_{5,62}$	rms error[d]	
										1	2
1	observed			6.2	4.8	7.1	5.3	9.5	3.3		
2	MM2	eq 1	56.3	5.8	7.0	8.1	6.0	9.4	3.6	1.05	1.54
3	MM2	eq 2	56.3	4.5	6.1	6.6	4.5	7.9	2.6	1.18	2.12
4	Chem3D	eq 1	58.7	5.7	6.8	8.5	5.8	9.6	3.4	1.02	1.55
5	Chem3D	eq 2	58.7	4.4	5.9	7.0	4.6	8.2	2.6	1.07	2.08
6	AM1	eq 1	93.6	6.4	3.1	11.2	6.5	12.4	4.1	2.25	2.64
7	AM1	eq 2	93.6	4.6	2.5	8.9	6.1	10.5	4.0	1.50	2.56
8	MNDO	eq 1	63.7	7.9	5.2	8.3	6.8	9.1	5.2	1.33	1.35
9	MNDO	eq 2	63.7	6.5	4.7	6.8	5.5	7.7	4.3	0.89	1.61

[a] The method used to calculate conformations of the structure. [b] Equation numbers as defined below. [c] The calculated total population in conformations with the 5-methoxy group in an equatorial position. [d] Standard error, the root mean square difference between calculated couplings for 1 and observed coupling constants for 1 and 2.

Table II. Observed and Calculated Vicinal Coupling Constants for Compound 2

entry	conf. calc[a]	Karplus eq[b]	amount eq[c] (%)	$J_{1,61}$	$J_{1,62}$	$J_{41,5}$	$J_{42,5}$	$J_{5,61}$	$J_{5,62}$	rms error[d]	
										1	2
1	observed			9.3	6.1	7.6	5.2	9.4	3.0		
2	MM2	eq 1	84.2	11.2	5.2	10.9	5.9	11.7	3.4	2.72	1.86
3	MM2	eq 2	84.2	9.8	4.6	8.8	5.2	9.9	3.0	1.63	0.83
4	Chem3D	eq 1	83.6	11.1	5.2	10.8	5.9	11.6	3.5	2.69	1.83
5	Chem3D	eq 2	83.6	9.7	4.6	8.7	5.2	9.9	3.0	1.61	0.82
6	AM1	eq 1	99.8	12.3	6.1	12.2	5.9	13.0	3.8	3.59	2.70
7	AM1	eq 2	99.8	10.7	5.3	9.8	5.7	10.8	3.7	2.24	1.31
8	MNDO	eq 1	55.1	7.6	6.8	7.5	7.5	8.3	5.3	1.66	1.59
9	MNDO	eq 2	55.1	6.7	5.8	6.4	6.2	7.1	4.2	1.23	1.62

[a] The method used to calculate conformations of the structure. [b] Equation numbers as defined below. [c] The calculated total population in conformations with the 5-methoxy group in an equatorial position. [d] Standard error, the root mean square difference between calculated couplings for 2 and observed coupling constants for 1 and 2.

calculation. It also takes into account different substitution patterns on the ethane fragment, bond angles of hydrogens, and Mullay[11] electronegativities of substituents.

$$^{3}J = 7 - \cos\theta + 5\cos 2\theta \qquad (1)$$

$$^{3}J = A\cos\theta + B\cos 2\theta + WE\cos\theta\sum\Delta\chi_i\cos\phi_i + H\{(\omega_1 + \omega_2)/2 - 110\} + M \qquad (2)$$

In these equations, θ is the dihedral angle between the hydrogens, while ϕ is a dihedral angle between one of the two coupling hydrogens and a substituent on the other carbon of the ethane fragment with Mullay electronegativity χ_i. $\Delta\chi_i$ is defined as $(\chi_i - \chi_H)$, where χ_H is the electronegativity of hydrogen (2.08).[11] The two angles between the C–C bond of the ethane fragment and the coupling hydrogens are called ω_1 and ω_2. In our case, where all ethane fragments are trisubstituted, the constants in eq 2 are $A = -1.3556$; $B = 4.9649$; $W = 1.41$; $E = 1.0374$; $H = -0.2061$; $M = 6.4068$. The calculation of Mullay electronegativities is presented in the supplementary material.

In order to permit a valid comparison with calculated coupling constants, the experimental data had to be determined with a reasonable accuracy. Because of the line broadening which was caused by extensive long-range couplings, this turned out to be nontrivial for compound 1. By simulation[12] using the well-resolved signal of H_5 to determine starting values, the coupling constants could be determined. The results, which are the experimental values of Tables I–IV, were verified by selective spin decoupling experiments.

The vicinal coupling constants were calculated for all low-energy conformations. These coupling constants were averaged by a weight factor proportional to the relative populations of conformations. In this way the coupling constants could be compared directly with the observed values. The results are presented in Tables I and II. One weakness with this procedure is that the populations are based on the calculated *enthalpies* for the conformations and not the free energies. Thus this procedure will only yield correct results when entropy differences are negligible between conformations. In order to overcome this limitation, linear combinations of the calculated coupling constants for the two major half-chair conformations were matched with observed coupling constants to the best fit, using least squares methods. The results are presented in Tables III and IV.

Calculation of NOE Effects. The expression for an NOE enhancement as a function of internuclear distances and other NOEs to the same spin when the molecule is in fast conformational equilibrium is given by eq 3 where $\langle\ \rangle$ denotes an average over all contributing conformations.[13] $f_I\{S\}$ is the observed steady-state enhancement at

$$f_I\{S\} = \eta_{\max}\left[\frac{\langle r_{IS}^{-6}\rangle - \sum_{X\neq I,S} f_X\{S\}\langle r_{IX}^{-6}\rangle}{\langle r_{IS}^{-6}\rangle + \sum_{X\neq I,S}\langle r_{IX}^{-6}\rangle + a_I}\right] \qquad (3)$$

spin I upon irradiation of spin S corrected for partial saturation, and r_{IS} is the distance between proton I and S. The denominator describes the relaxation of spin I.

---

(11) (a) Mullay, *J. Am. Chem. Soc.* **1984**, *106*, 5842–5847. (b) Mullay, J. *J. Am. Chem. Soc.* **1985**, *107*, 7271–7275. (c) Mullay, J. *J. Am. Chem. Soc.* **1986**, *108*, 1770–1775.

(12) *Panic* on a Bruker Aspect 3000.

(13) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers, Inc.: New York, 1989.

Table III. Variation of the Amount of Isomers with an Equatorial 5-Methoxy Group, Compound 1

entry	conf. calc[a]	Karplus eq[b]	amount eq[c] (%)	$J_{1,61}$	$J_{1,62}$	$J_{41,5}$	$J_{42,5}$	$J_{5,61}$	$J_{5,62}$	error[d] (Hz)
1	observed			6.2	4.8	7.1	5.3	9.5	3.3	
2	MM2	eq 1	61.2	5.8	6.5	8.6	6.0	9.8	3.6	1.00
3	MM2	eq 2	71.8	4.5	4.8	7.7	5.0	9.0	2.9	0.65
4	Chem3D	eq 1	61.1	5.8	6.6	8.7	5.8	9.8	3.4	1.02
5	Chem3D	eq 2	69.7	4.4	4.9	7.8	4.9	9.0	2.8	0.74
6	AM1	eq 1	63.4	6.4	6.0	8.3	6.7	9.6	4.3	0.96
7	AM1	eq 2	70.0	4.9	4.5	7.2	5.5	8.7	3.6	0.42
8	MNDO	eq 1	63.9	7.9	5.2	8.3	6.8	9.2	5.2	1.78
9	MNDO	eq 2	72.7	6.4	4.0	7.4	5.7	8.4	4.4	0.57
10	MM2(2)	eq 1	45.2	7.4	5.5	7.0	6.3	8.5	3.7	0.68[e]
11	Chem3D(2)	eq 1	45.2	7.4	5.5	6.9	6.4	8.5	3.7	0.70[e]

[a] The method used to calculate conformations of the structure. [b] Equation numbers as defined above. [c] The population in conformations with the 5-methoxy group in an equatorial position that gave the best fit to experimental data. [d] Standard error, the rms difference between calculated and observed couplings. [e] Populations varied to fit the calculations for compound **2** to the observed values for compound **1**.

Table IV. Variation of the Amount of Isomers with an Equatorial 5-Methoxy Group, Compound 2

entry	conf calc[a]	Karplus eq[b]	amount eq[c] (%)	$J_{1,61}$	$J_{1,62}$	$J_{41,5}$	$J_{42,5}$	$J_{5,61}$	$J_{5,62}$	error[d] (Hz)
1	observed			9.3	6.1	7.6	5.2	9.4	3.0	
2	MM2	eq 1	58	8.6	5.4	8.2	6.2	9.5	3.6	0.44
3	MM2	eq 2	76	9.0	4.5	8.1	5.0	9.3	2.9	0.47
4	Chem3D	eq 1	58	8.7	5.4	8.2	6.2	9.5	3.6	0.45
5	Chem3D	eq 2	76	9.1	4.5	8.1	5.0	9.3	2.9	0.48
6	AM1	eq 1	63	8.7	6.1	8.5	6.6	9.5	4.1	0.75
7	AM1	eq 2	79	8.9	5.1	8.2	5.5	9.3	3.4	0.29
8	MNDO	eq 1	68	8.9	6.7	8.8	7.1	9.7	5.0	1.61
9	MNDO	eq 2	80	8.8	5.9	8.4	6.2	9.1	4.2	0.57
10	MM2(1)	eq 1	59	5.8	6.7	8.4	6.0	9.7	3.6	2.35[e]
11	Chem3D(1)	eq 1	59	5.7	6.8	8.5	5.8	9.6	3.4	2.42[e]

[a] The method used to calculate conformations of the structure. [b] Equation numbers as defined above. [c] The population in conformations with the 5-methoxy group in an equatorial position that gave the best fit to experimental data. [d] Standard error, the rms difference between calculated and observed couplings. [e] Populations varied to fit the calculations for compound **1** to the observed values for compound **2**.

Table V. Calculated Steady-State NOE Effects for Structures 1 and 2 Using eq 5 (Conformations from MM2, Populations from Enthalpies Only)

structure 1, rms error = 1.1%							structure 2, rms error = 2.1%						
$f_I\{S\}$	H_1	H_{41}	H_{42}	H_5	H_{61}	H_{62}	$f_I\{S\}$	H_1	H_{41}	H_{42}	H_5	H_{61}	H_{62}
$\{H_1\}$		0.1	0.0	0.3	5.5	1.0	$\{H_1\}$		0.1	−0.2	6.2	−0.3	5.4
$\{H_{41}\}$	0.1		32.7	1.2	2.3	−1.2	$\{H_{41}\}$	0.3		31.8	−0.3	4.0	−1.3
$\{H_{42}\}$	0.1	34.7		9.1	−1.3	2.0	$\{H_{42}\}$	−0.6	35.4		9.3	−1.3	0.7
$\{H_5\}$	0.5	0.7	5.1		1.3	3.8	$\{H_5\}$	9.7	−0.2	5.3		0.6	3.4
$\{H_{61}\}$	13.8	2.3	−1.2	2.2		35.8	$\{H_{61}\}$	−0.6	3.7	−1.1	0.8		34.8
$\{H_{62}\}$	2.5	−1.2	1.9	6.3	34.7		$\{H_{62}\}$	13.8	−1.3	0.6	5.4	38.5	

Table VI. Calculated Steady-State NOE Effects for Structures 1 and 2 Using eq 5 (Conformations from MM2, Populations from Tables III and IV (Entry 3 in Both Tables))

structure 1, rms error = 1.0%							structure 2, rms error = 1.8%						
$f_I\{S\}$	H_1	H_{41}	H_{42}	H_5	H_{61}	H_{62}	$f_I\{S\}$	H_1	H_{41}	H_{42}	H_5	H_{61}	H_{62}
$\{H_1\}$		0.0	0.0	0.4	5.6	1.4	$\{H_1\}$		0.1	−0.2	5.5	0.0	5.4
$\{H_{41}\}$	0.1		32.3	0.4	2.8	−1.1	$\{H_{41}\}$	0.3		31.9	−0.1	3.6	−1.2
$\{H_{42}\}$	0.1	35.0		9.8	−1.2	1.3	$\{H_{42}\}$	−0.5	35.3		9.2	−1.3	0.9
$\{H_5\}$	0.5	0.2	5.2		0.8	3.9	$\{H_5\}$	8.7	0.0	5.3		0.8	3.3
$\{H_{61}\}$	13.5	2.8	−1.1	1.4		35.6	$\{H_{61}\}$	−0.1	3.3	−1.1	1.2		34.7
$\{H_{62}\}$	3.4	−1.1	1.2	6.7	35.1		$\{H_{62}\}$	13.7	−1.2	0.9	5.4	38.0	

The *r* terms result from intramolecular dipolar relaxation to other protons, while the constant a_I describes all other contributions to the relaxation of spin *I*. The constant $\eta_{\max}$ is 50% at the extreme narrowing limit. Similarly, the expression for direct NOEs is shown in eq 4.

$$f_{I,0}\{S\} = \eta_{\max}\left[\frac{\langle r_{IS}^{-6}\rangle}{\sum_{X \neq I} \langle r_{IX}^{-6}\rangle + a_I}\right] \tag{4}$$

The added index 0 is our notation for the direct contribution to the NOE. Note that the spin *S* is included in the sum. The internuclear distances *r* were calculated from the MM2 structures. The results in Table V are obtained using populations calculated from enthalpies. An NOE version of the variation method described above is very hard to implement, due to the complexity of the calculation. However, the populations used to obtain the data in Tables III and IV were also used to calculate NOEs. The results of these calculations are shown in Table VI.

One problem is that eq 4 is not valid for methyl protons. For example, $\langle r^{-3}\rangle^2$ should be used in place of the $\langle r^{-6}\rangle$ terms because of the fast rotation of the methyls. Other changes are introduced due to the magnetic equivalence of the methyl protons. Therefore no NOEs were calculated to or from methyl groups, but distances to the methyl

Table VII. Some Calculated Steady-State NOE Effects for Structures 1 and 2 Using eq 5[a]

	structure 1			structure 2		
	Chem3D	AM1	MNDO	Chem3D	AM1[b]	MNDO
$f_{H_5}\{H_1\}$	0.6	0.6	0.4	9.9	10.2	3.3
$f_{H_{61}}\{H_1\}$	6.2	7.1	8.0			
$f_{H_{62}}\{H_1\}$	0.9	1.7	−0.3	5.8	6.6	6.9
$f_{H_5}\{H_{41}\}$	0.4	−4.1	−1.6			
$f_{H_5}\{H_{42}\}$	15.3	19.9	16.6	14.4	15.9	16.9
$f_{H_1}\{H_5\}$	1.0	0.8	0.6	14.9	14.9	5.4
$f_{H_{41}}\{H_5\}$	0.2	−1.9	−0.8			
$f_{H_{42}}\{H_5\}$	6.7	8.6	7.7	7.1	7.7	8.9
$f_{H_{61}}\{H_5\}$	1.2	−0.5	0.0			
$f_{H_{62}}\{H_5\}$	4.2	5.9	5.8	3.6	4.1	5.0
$f_{H_1}\{H_{62}\}$	3.5	5.6	−0.9			
$f_{H_5}\{H_{62}\}$	10.2	14.6	14.0			
rms error	2.3	4.4	3.6	5.3	5.8	4.7

[a] Two representative conformations from each calculated set, scaled to represent the relative amount of isomers with equatorial and axial 5-methoxy, methyls ignored. [b] Two diequatorial conformations were used.

protons were still included in the denominator to refine the calculation of NOEs between ring protons.

We also performed a simplified calculation based upon conformations from Chem3D, AM1, and MNDO. Here only the six ring protons of structures 1 and 2 were included. The relaxations to the methyl groups were combined with other unknown leakage factors into a_I. Each of these calculations was performed using only two representative conformations from each set of structures, one with an equatorial and one with an axial 5-methoxy group. The populations from Tables I and II were used for the averaging of these representative conformations. In the AM1 calculation for structure 2, two diequatorial conformations were used with their relative enthalpy based populations. These results are shown in Table VII.

The direct NOEs were obtained from the calculated structures using eq 4. In order to calculate the steady-state NOEs eqs 3 and 4 were combined setting $f_S\{S\} = -1$ (the basis of compensation for incomplete saturation) and assuming that a_I could be neglected. The resulting eq 5 was then solved for all combinations of I and S. When only the six ring protons are considered, this gives a total of 30 equations which could be divided into six equation systems of five equations each. However, including relaxation to the methyl protons in the calculation of NOEs between ring protons resulted in six equation systems of 17 equations each.

$$f_I\{S\} + \sum_{X\neq I} f_X\{S\} f_{I,0}\{X\} = 0 \qquad (5)$$

$$\left\{\begin{array}{ll} f_{I,n+1}\{S\} = -\sum_{X\neq I} f_{X,n}\{S\} f_{I,0}\{X\} & I \neq S \\ f_{S,n+1}\{S\} = -1 & \end{array}\right\} n = 0, 1, \ldots \qquad (6)$$

Alternatively, an iterative method based on stepwise perturbation of the initially calculated direct NOEs ($f_{I,0}$) could be used (eq 6). This method does not involve solution of equation systems, which makes it advantageous for large systems.

Results and Discussion

With both diastereoisomers 1 and 2 available, it is easy to assign their relative stereochemistry based on coupling constants and NOEs. The most conclusive evidence is the diaxial couplings from H_1 and H_5 to H_{61} for the isomer 2 ($J_{1,61}$ = 9.3 Hz, $J_{5,61}$ = 9.4 Hz, Table II). The relative stereochemistry of H_1 and H_5, trans in 1 and cis in 2, is also shown by the NOEs which are weak for 1 ($f_{H_5}\{H_1\}$ and $f_{H_1}\{H_5\} < 1$) and strong for 2 ($f_{H_5}\{H_1\} = 4$, $f_{H_1}\{H_5\} = 7$, Tables V and VI). The trans configuration of 1 is also indicated independently by the mass spectra, which show a peak at M-15 for 1 only. This can be attributed to a MacLafferty abstraction of H_5 by the carbonyl, followed by loss of the 5-methoxy methyl group, a reaction only possible in the trans isomer.

In contrast, assignments based on the comparison of experimental data for only 1 with the computed observables for both isomers are not as straightforward. One obvious problem is that the different computational methods give somewhat different results. This is also shown in a recent study comparing conformational energies calculated by several molecular mechanics and semiempiric methods.[14]

In our calculations for the compound 1, both molecular mechanics methods gave nine distinct minima with an equatorial 5-methoxy group and four with an axial methoxy group. All the conformations are half-chairs. The best equatorial and axial conformers have about the same calculated energy. For the compound 2, six minima with diequatorial and six with diaxial substituents were obtained. The differences between the minima are small for both compounds 1 and 2 and caused partly by the rotation of the methoxy group. The methoxycarbonyl group generally prefers a conformation where it is eclipsed with the C_1–H_1 bond. The two most stable conformations of compound 1 are shown in Figure 2.

The AM1 package in Mopac gave similar results in that for compound 1 eight minima with an equatorial and four with an axial methoxy group were obtained, and for 2 six minima of each kind were obtained. The MNDO package, in contrast, gave four minima with similar energies for each of the two possible half-chair conformations for both isomers 1 and 2. In the preferred conformations, the methoxy group is eclipsed with H_5.

Coupling constants were calculated for each minimum energy conformation, using eqs 1 and 2. In order to compare with experimental values, an average based on Boltzmann distribution was estimated by approximating free energies by enthalpies.

One major difference between the molecular mechanics and semiempirical methods is the calculated energy

---

(14) Gundertofte, K.; Palm, J.; Pettersson, I.; Stamvik, A. *J. Comput. Chem.* **1991**, *12*, 200–208.

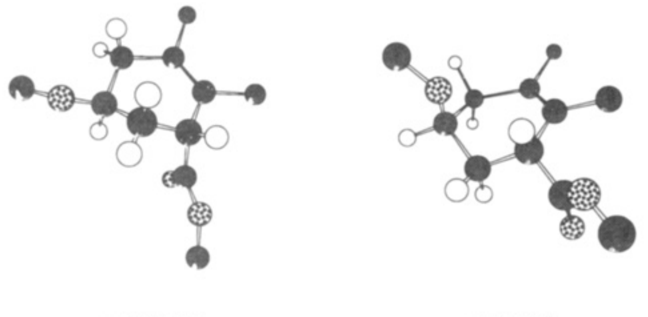

equatorial 5-MeO axial 5-MeO

Figure 2. The two most stable half-chair conformations of compound 1. Methyl hydrogens are removed for clarity.

difference between equatorial and axial conformations of the methoxy group. Both MM2 and Chem3D calculate that at equilibrium the 5-methoxy group is ca. 60% equatorial for **1** and 80% equatorial for **2** (Tables I and II), which intuitively seems very reasonable. The experimental values agree well with the calculated which have mean standard errors of ca. 1 Hz for both **1** and **2** (Tables I and II). The predictions by MNDO yield similar populations for **1**, but for **2** essentially equal populations are predicted for the diequatorial and the diaxial conformations (55/45). This would indicate a contribution from weak attractive interaction between the methoxy and the carbonyl group in **2**. However, **2** and **1** give about the same rather poor fit, standard error ca. 1.5 Hz, to the data for **2**, indicating that this is an artefact from the calculation. By contrast, AM1 appears to overestimate the relative bulk of the 5-methoxy group and predicts predominant (93%) equatorial conformation for **1** and complete (99.8%) diequatorial conformation for **2**. A relatively poor fit to the experimental data also for this method (entries 6 and 7, Tables I and II) suggests that the predictions from the molecular mechanics calculations are more reliable.

When the calculated and experimental coupling constants are compared, it is clear that for the isomer **2** in particular, the more complex eq 2 gives a better fit. It is also evident that the molecular mechanics, but not the semiempirical methods, are able to predict correctly the stereochemistry of **1** and **2** by comparing the calculated coupling constants for both with the experimental data for only one of them. However, even with these methods, the fit is not as good as one might wish. The obvious reason is that the energy calculations are not sufficiently accurate, as also suggested in a recent paper, presenting an elaborate fitting of conformational populations and NOE data.[15] In order to overcome this problem, the entropies of the different conformations must be estimated. This can be done in different ways: (1) The energy profile of a conformation is calculated using dihedral drivers. A flat, wide minimum means a high entropy value, which can be estimated by dividing the potential energy well into small sections and calculating the population of each section from enthalpies. (2) The entropies are estimated by comparison between calculated and experimental data. In this work, both methods were investigated in a qualitative way. By dihedral driver calculations it could be shown that the minima are wider for conformations with an equatorial methoxy groups. This means that the real populations with an equatorial methoxy group should be higher than suggested from calculated enthalpies but probably not as high as by the AM1 method.

In order to simplify the implementation of method 2, it was assumed that all conformations with the same main conformation of the ring have the same entropy. This reduces the problem to a determination of the relative populations of the two main conformations of each isomer. This was done by treating the total amount of conformers with equatorial 5-methoxy group as a variable and determining its value by a least squares fit of the calculated coupling constants to the experimental results. The results of these calculations are shown in Tables III and IV.

The use of eq 2 gives higher preference for equatorial methoxy groups and distinctly better rms values. In agreement with the dihedral driver calculations, the relative importance of the conformations of **1** with equatorial methoxy group has increased to ca. 70% when eq 2 is used in combination with molecular mechanics calculations. It is reassuring that both semiempirical also give about the same value. All methods give very small standard errors (0.42–0.74 Hz). The values are now slightly better for the semiempirical methods, suggesting that these give very good structures but fairly poor estimates of energies.

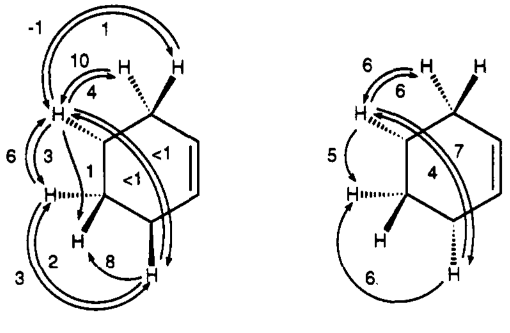
Also for compound **2**, the four methods agree about the conformation, giving ca. 80% in diequatorial conformations. Again standard errors are very small (0.29–0.57 Hz). An interesting feature is that the molecular mechanics methods, in contrast to the semiempirical methods, give the wrong order among the two coupling constants $J_{1,61}$ and $J_{1,62}$ for compound **1**. This is true for the variation method as well as direct calculation (Tables I–IV). One reason could be that the hybridization of C_1 has partial sp^2 character due to both an adjacent double bond and a carbonyl function. This illuminates one of the major differences between the molecular mechanics and semiempirical approaches to structure determination. The much larger set of variable parameters make the molecular mechanics calculations more exact, but only when the molecules are very similar to the ones for which parameterization has been done. When a molecule contains an atom type that has not been included in the parameterization, errors occur.

While the variation method (Tables III and IV) gives improved fit to the observed coupling constants, it can also be seen that structure **2** with a dominant (55%) diaxial conformation gives a better fit to the experimental values for compound **1** than **1** itself (entries 10 and 11, Table III). Although a preference for a diaxial conformation for **2** appears to be against chemical intuition, it should be noted that only small energy differences are involved. A small attractive interaction between the methoxy and carbonyl groups would be sufficient and in fact, the figure 55% diaxial is very close to that predicted by MNDO (45%, entries 8 and 9, Table II). In contrast, the calculated data for structure **1** could not be fitted to the data for compound **2** (entries 9 and 10, Table IV). The results clearly suggest that the variation method should be used with caution.

It thus appears that coupling constants can be used together with molecular mechanics calculations to distinguish between isomeric cyclohexenes with a fair accuracy. It is not clear if this conclusion can be generalized, but this seems possible. In contrast, the semiempirical methods give fairly high standard errors both for **1** and **2** (Tables I and II) and the differences when structures **1**

---

(15) Landis, C.; Allured, V. S. *J. Am. Chem. Soc.* **1991**, *113*, 9493–9499.



1 2

Figure 3. Observed steady-state NOE in compounds 1 and 2.

and 2 are fitted to the data for compounds 1 are not conclusively different. Use of the variation methods (Tables III and IV) suggest that the reason is poor energy calculations by these methods. The general predictive value of these methods seems uncertain. It therefore seemed important to investigate also the comparison of calculated and experimental NOEs.

In Figure 3 the important experimental NOEs for 1 and 2 are summarized. All calculated steady-state NOEs are shown in Tables V–IX. The rms values are calculated using only the effects shown in Figure 3. Because 2 is only ca. 80% pure, overlapping signals and noise make determination of small effects difficult and only a moderate amount of experimental data could be determined as shown in Figure 3.

The calculated direct NOEs, which are obtained from eq 4, are proportional to the initial buildup rate of the NOE measurable with techniques like NOESY and truncated NOE (TOE). However, for small molecules it is usually more informative to use these values to calculate the steady state NOE expected at long irradiation times, using eqs 5 or 6. In the present study, the irradiation time was 12 s which corresponds to about $2T_1$ for the proton with the longest relaxation time (H_1) and ca. $5T_1$ for the other protons. Table V shows the NOEs calculated from all MM2 conformations weighted according to their enthalpy based populations.

The variation method used above indicates that improvements can be gained by adjusting the relative populations of the main conformations. Therefore we also changed the populations of all conformations to conform to Table III, entry 3, for 1 and Table IV, entry 3, for 2. The results of this calculation is shown in Table VI. For example, the strongest observed nongeminal NOE in compound 1 ($f_{H_5}\{H_{42}\}$, 10%) is well reproduced already by the calculations in Table V (9.1%) and perfectly in Table VI (9.8%). For compound 2, the axial–axial NOE $f_{H_1}\{H_5\}$ (observed 7%) is calculated to 9.7% in Table V and improved to 8.7% when the population variation is implemented in Table VI.

It can be seen from Tables V and VI that the correspondence is very good when all protons in the molecule are included in the calculation (standard errors 1.0–2.1). The fact that the error goes down when the populations from Tables III and IV are used gives another indication that the variation method is useful for refining the relative populations of isomers. The main errors come from the ignored leakage factor a_I, making the calculated NOEs a little too high. Even with the most accurate method (Table VI), in one case ($f_{H_{61}}\{H_1\}$, compound 1) the calculated value (5.6%) is substantially lower than the observed value (8%). This again indicates that carbon 1 experiences a slight rehybridization that is not accounted for by the molecular mechanics force fields. For compound 2, the calculations seem slightly less accurate. This may be due to either the inability of MM2 to account for the possible interaction between the 5-methoxy oxygen and the carbonyl carbon, or the larger experimental error for 2.

In the simplified calculations (Table VII), the calculated NOEs are usually higher than the experimental values due to the ignored leakage factor a_I. It is interesting to note that both semiempirical methods give essentially correct values for $f_{H_{61}}\{H_1\}$ in compound 1 (observed: 8%), whereas Chem3D, like the accurate MM2 calculation above, gives a low calculated value. The largest discrepancies between calculated and observed NOEs can be seen in effects to H_1 and H_5 in compound 2. Here the experimental NOEs are much lower than the calculated values. This is expected, since these protons without close neighbors have a large portion of their relaxation to the methyl groups. This is true to a lower extent for H_5 in compound 1. A little surprisingly, MNDO calculates accurate values for $f_{H_5}\{H_1\}$ and $f_{H_1}\{H_5\}$ in compound 2 (observed: 4% and 7%). We believe this is an artefact due to a cancellation of two errors: exclusion of the methyls (raising the calculated NOE, see above) and a high calculated population in the diaxial conformer (with a long distance between H_1 and H_5).

The r^{-6} relationship between distance and relaxation should in principle introduce large uncertainties in calculations of NOE, since any error in calculated internuclear distances would also be raised to the sixth power. Also, if the internuclear distance is short enough in a specific conformation, this doe not need to be an energy minimum to make a sizeable contribution to the observed NOE. Considering these sources of error, the fit of calculated steady state to observed NOEs is remarkable for dynamic systems like these. This again indicates that structures are calculated very well by the methods used here. No attempt was made to refine the populations using NOE data (cf. the variation method used for coupling constants). In principle, this is possible, but each variation of the relative populations requires a new solution of either eq 5 or eq 6. This precludes the use of the simple least squares fit used above.

Regarding the stereochemical assignments of 1 and 2 the NOE data are completely consistent. For 1, the NOEs in Figure 3 clearly show that H_5 is cis to H_{42} and H_{62}, whereas H_1 is cis to H_{61}. For 2, the all-cis relationship between H_1, H_{62}, H_5, and H_{42} is evident.

The calculation of direct NOEs according to eq 4 is fairly simple while the steady-state NOEs require the solution of eq 5. For compounds 1 and 2 excluding methyl protons this means solving six equation systems of five equations each which is not very difficult. However, for systems involving many atoms, an iterative solution could be more favorable. We therefore also calculated the steady-state NOEs from direct NOEs by the eq 6, using the simplified NOEs from Chem3D structures as an example. In each iterative step, the convergence was measured by calculating the rms deviation of all calculated NOEs (not just the experimentally measured ones) from the known analytic solution. Already after the first iteration, the largest error was 1.2% and the rms error was 0.11%. After five iterations the two sets of calculated NOEs were essentially identical (rms error <0.01%). The details of this calculation are available in the supplementary material.

In conslusion, molecular mechanics calculations, in combination with NMR, is an excellent method for establishing the stereochemistry of compounds such as cyclohexenes, in which several conformations are in fast equilibrium. Both in calculations of vicinal coupling constants and NOEs, molecular mechanics methods (MM2, Chem3D) give better results than semiempirical quantum chemical methods (AM1, MNDO), probably due to a more accurate steric energy calculation, but with some exceptions the results are reasonable with all methods. In the present case, both methods are able to distinguish between the stereoisomers 1 and 2, but the combination of molecular mechanics and steady-state NOE calculation is clearly the superior method.

Experimental Section

NMR spectra were run on a Bruker AM 400 in $CDCl_3$ or DMSO-d_6. NOE samples were fitted with a rubber septum, thoroughly degassed by three freeze–thaw cycles under vacuum, and filled with argon. Irradiation time in NOE runs was 12 s. The preparation of compound 1 has been described previously.[1] GC–MS were run on a Finnigan 4500 mass spectrometer.

***trans*-Methyl 2,3-Dimethyl-5-methoxy-2-cyclohexene-1-carboxylate (1).** ^{1}H NMR: δ (DMSO-d_6) 1.56 (m, 3 H, Me_2, $J_{1,2} = 1.0^a$ Hz, $J_{2,3} = 0.9$ Hz, $J_{2,4} = 1.9$ Hz), 1.59 (m, 3 H, Me_3, $J_{1,3} = 1.8$ Hz, $J_{3,4} = 0.9^a$ Hz), 1.66 (ddd, 1 H, H_{61}, $J_{1,61} = 6.2$ Hz, $J_{5,61} = 9.5$ Hz, $J_{61,62} = 12.8^a$ Hz), 1.86 (m, 1 H, H_{41}, $J_{1,41} = 1.7$ Hz, $J_{41,42} = 17.0^a$ Hz, $J_{41,5} = 7.1$ Hz), 1.98 (dddd, 1 H, H_{62}, $J_{1,62} = 4.8$ Hz, $J_{42,62} = 1.4^a$ Hz, $J_{5,62} = 3.3$ Hz), 2.27 (m, 1 H, H_{42}, $J_{1,42} = 1.4$ Hz, $J_{42,5} = 5.3$ Hz), 3.08 (m, 1 H, H_1, $J_{1,5} \leq 0.3^a$ Hz), 3.23 (s, 3 H, MeO_5), 3.56 (dddd, 1 H, H_5), 3.61 (s, 3 H, $MeOCO_1$) [couplings marked with a superscript "a" are probably negative (geminal, isopropylidenic and W)]. In $CDCl_3$, the shifts of H_5, H_{61}, and H_{62} were about 0.05 ppm higher. The effect on other shifts and couplings were insignificant. MS: *z/e* 198 (M^+, 0.7), 183 (M − 15, 0.8), 166 (63), 139 (24), 125 (17), 107 (100), 91 (31), 79 (21), 58 (21), 45 (28), 41 (27).

***cis*-Methyl 2,3-Dimethyl-5-methoxy-2-cyclohexene-1-carboxylate (2).** A small piece of freshly cut sodium was added to 10 mL of dry methanol. When the reaction was complete, one drop of compound 1 was added. After 1 h, the methanol was evaporated and the residue extracted with dry ether. The ether was evaporated, and the resulting yellowish oil was purified by HPLC using petroleum ether/ethyl acetate (90/10) as eluent. The resulting clear oil was a mixture of compound 1 and 2. It was further enriched using the same HPLC system to an 20/80 mixture of 1 and 2 (by NMR). All further spectra were run on this sample. ^{1}H NMR: δ ($CDCl_3$) 1.62 (m, 3 H, Me_2), 1.66 (m, 3 H, Me_3), 1.90 (dt, 1 H, H_{61}, $J_{1,61} = 9.3$ Hz, $J_{5,61} = 9.4$ Hz, $J_{61,62} = 12.4$ Hz), 2.10 (m, 1 H, H_{41}, $J_{1,41} \approx 2.5$ Hz, $J_{41,42} = 17.0$ Hz, $J_{41,5} = 7.6$ Hz), 2.13 (dddd, 1 H, H_{61}, $J_{1,62} = 6.1$ Hz, $J_{42,62} = 1.6$ Hz, $J_{5,62} = 3.0$ Hz), 2.26 (m, 1 H, H_{42}, $J_{1,42} \approx 1.5$ Hz, $J_{42,5} = 5.2$ Hz), 3.08 (m, 1 H, H_1), 3.33 (s, 3 H, MeO_5), 3.41 (dddd, 1 H, H_5), 3.70 (s, 3 H, $MeOCO_1$). MS: *z/e* 198 (M^+, 0.2), 166 (19), 139 (10), 125 (9), 107 (100), 91 (18), 84 (18), 79 (11), 58 (9), 49 (30), 45 (14), 41 (17).

Acknowledgment. We are grateful to Professor T. Liljefors at The University of Lund, for valuable help with MM2, and to Dr. R. Carter for initial help in this project. We also thank Professor E. Osawa for disclosure of material prior to publication and for pointing out an assignment error in our preliminary communication. Financial support from The Swedish National Science Research Council is gratefully acknowledged.

Supplementary Material Available: Calculation of electronegativities used in eq 2, the calculated steady-state NOEs abbreviated in Table VII, and the calculated direct NOEs and subsequent refinement to steady-state NOEs according to eq 6 (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.