Table III. K_A Values for Binding of Iodovancomycin and Iodinated Ristocetin Derivatives to Ac_2-L-Lys-D-Ala-D-Ala[a]

compound	K_A
vancomycin (1)	$1.5 \pm 0.1 \times 10^6$
iodovancomycin (3)	$3.4 \pm 0.3 \times 10^5$
ristocetin (2)	$2.6 \pm 0.1 \times 10^5$
iodoristocetin (8)	$1.8 \pm 0.1 \times 10^5$
Ψ-AGIR (9)	$1.7 \pm 0.2 \times 10^5$
Ψ-AGR (10)	$3.4 \pm 0.6 \times 10^5$
I-Ψ-AGR (11)	$2.1 \pm 0.2 \times 10^5$

[a] K_A values were determined by UV difference spectroscopy at 298 K. Solutions were prepared at pH 5.1 in 0.02 M sodium citrate buffer.

pseudoaglycon of iodoristocetin (Ψ-AGIR, 9); by HPLC it was identical with the minor constituent seen in crude 9.

To facilitate the structure assignment of the new iodinated pseudoaglycon, designated at I-Ψ-AGR, a comparison with 9 and 10 was made by NMR (Table II), with particular attention being paid to the aromatic region.

The NMR spectrum of iodinated isomer, I-Ψ-AGR, was found to be remarkably similar to that of 10 with the exception of the signals of ring 7. Analogous to the case of vancomycin, a new peak is present at 6.28 ppm in the spectrum of I-Ψ-AGR which has been assigned as 7f on the basis of an NOE observed to the α-proton of residue 7. Ring 3 is essentially unchanged. Removal of the mannose, therefore, causes the site of iodination to shift from predominantly 3b to 7d, the same site as in vancomycin, and the structure of I-Ψ-AGR can be assigned as 11.

Chemical degradation of 9 and 11 was carried out as for vancomycin. From the degradation of crude 9 a trace of bis(nitrile) 6 was isolated, indicating that iodination, albeit very slowly, occurs to some extent on ring 7 in the parent antibiotic. No product from iodination of ring 3 (such as bis(nitrile) 12) could be isolated; possibly it could not survive the highly basic hydrolysis conditions. We think it is more likely, however, that the iodination product escapes detection because the iodo substituent sterically inhibits both methylation of the phenol and hydrolysis of the peptide bond. Degradation of 11 gave the expected bis(nitrile) 6.

Peptide Binding. To determine if iodination causes changes in the affinity of these modified antibiotics for peptides, association constants for the binding of the tripeptide, Ac_2-L-Lys-D-Ala-D-Ala, were measured by UV difference spectroscopy (Table III). For vancomycin it can be seen that iodination causes a modest (less than an order of magnitude) decrease in binding affinity, which is in accord with the earlier results of Nieto and Perkins.[4b] In the current model for the tripeptide–antibiotic complex (in aqueous media) the lysyl side chain lies over ring 7;[6f,8] iodine at position 7d may perturb this orientation, leading to less effective binding. A similar but less marked effect is seen with ristocetin and its iodinated derivatives—the K_A for 8 is slightly less than that for 2 and compounds 9 and 11 bind less effectively than 10 but overall, the effect of iodination is relatively minor.[15] Examination of molecular models reveals that neither iodination at 3b, as in 8 and 9, nor iodination at 7d, as in 11, significantly alters the binding pocket.

Acknowledgment. FAB spectra and exact mass measurements were obtained with the help of Dr. K. Tomer at the Midwest Center for Mass Spectrometry, Lincoln, NE (Grant No. CHE 8211164 from the National Science Foundation), and Brian Sweetman, Dept. of Pharmacology, Vanderbilt University (Grant No. GM 27557 from the USPHS). Funds for the support of this work were provided by the U.S. Public Health Service (GM 25793).

(15) The adjective "minor" used to describe the difference in K_As may be applicable in a chemical sense but not when translated into biological activity. When assayed for antibiotic activity against *B. subtilis*,[16] compound 3 showed about 50% the activity of the parent compound; i.e., twice the quantity of 3 is required for the same area of inhibition. This reduction in activity is roughly comparable in magnitude to the decrease seen in K_A but from a therapeutic viewpoint may be considered a major decrease. A strict correlation between binding constants and antibiotic activity would not be expected, given the complexity of the interaction between antibiotic and microorganism.

(16) Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Antibiot.* **1985**, *38*, 51–57.

Molecular Mechanics Studies on the Supra Annular Effect of 3-Cyclohexene-1-carboxaldehyde Compounds

Phillip Bowen and Norman L. Allinger*

Department of Chemistry, University of Georgia, Athens, Georgia 30602

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There is little experimental information available on the conformations of Δ^3-cyclohexene compounds. In the early sixties, it was proposed based on models that these systems adopt a conformation with the electronegative group axial. The stability of this arrangement was attributed to an intramolecular carbonyl–double bond interaction. Examining those structures with MMP2 force field and ab initio calculations, we have determined that this resonance effect is unimportant, and in fact there is no preference for an axial aldehyde group.

There has been a vast amount of stereochemical and conformational data obtained for cyclohexane derivatives.[1] The corresponding cyclohexene compounds, however, have not received as much attention.[1] In fact, little work has been reported on the 3-cyclohexene-1-carboxaldehyde series.[2,3] The purpose of this study was (1) to establish the

(1) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. "Conformational Analysis"; American Chemical Society, Washington, DC, 1981.

(2) Kugatova-Shemyakina, G. P.; Ouchinnikov, Yu. A. *Tetrahedron* **1962**, *18*, 697. Kugatova-Shemyakina, G. P.; Nikolaev, G. M.; Andreev, V. M. *Tetrahedron* **1967**, *23*, 2721.

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Table I. Distances (Å) between Atoms in 3-Cyclohexene-1-carboxaldehydes 2 and 3

compd	C(7)/C(3)	C(7)/C(4)
2	3.215	3.389
3	3.879	4.344

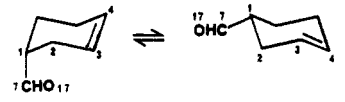
most favored conformations, (2) to compare the energetics of the various conformers, and (3) to establish quantitatively the extent of the resonance interaction between the carbonyl and double-bond moieties, if any.

Interesting work on 3-cyclohexene-1-carboxaldehyde and related compounds appeared in the literature some years ago by Kugatova-Shemyakina and co-workers.[2] They proposed that the observed reduced reactivities of both the double bond and carbonyl group were due to intramolecular interactions between these two functionalities and should be termed the *supra annular* effect.[2] They proposed that molecules of the general structure **1**, in order

1

to achieve this nonbonding overlap, would be forced to adopt a conformation with Z (electron-withdrawing group) axial. Models indicated that this was reasonable.

We began our study by examining the simplest case of 3-cyclohexene-1-carboxaldehyde itself. We decided to employ the following stratagem: We would determine the most stable conformation and energy with MMP2,[4] initially ignoring the overlap (in a quantum mechanical sense) between all nonbonded carbons. Another calculation was then carried out using the same geometries, with overlap between the carbonyl and double bond carbons explicitly included in the SCF routine. The energy difference between these two calculations corresponds to the energy stabilization due to the nonbonded overlap. Intuitively, we would indeed expect compound **2** to be stabilized more than **3**, for the distance between the carbonyl carbon, C-7,

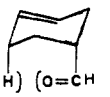


2 **3**

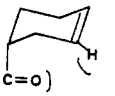
and the alkene carbons, C-3 and C-4, is greater in the latter (see Table I). But overlap falls off rapidly with distance, and, again intuitively, we would expect the advantage of **2** over **3** from this effect to be small.

There are three possible minimum energy conformers, corresponding to the carbonyl oxygen eclipsing either the hydrogen atom of C-1 or the ring carbons C-2 or C-6. These three rotamers are defined respectively as CONI, CONII, and CONIII. Depending upon the ring's substitution pattern, the most stable structure usually adopts either CONII or CONIII. Typically CONI has the highest energy conformation, and does not contribute significantly to the average energy.

It seemed reasonable for aldehyde **2** to prefer CONII because of the smaller van der Waals repulsion between the carbonyl oxygen and the C-3 hydrogen, as opposed to the greater van der Waals interactions with the C-5 axial



CONIII

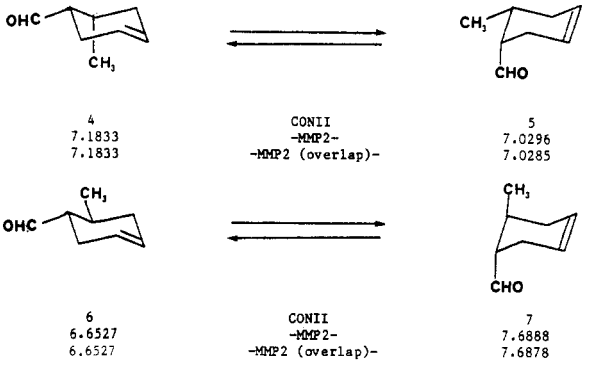


CONII

hydrogen of CONIII (see Table II).

Table IV indicates that there is only a 1.3-calorie energy stabilization due to the nonbonded overlap. Clearly, this miniscule energy difference is insufficient to cause the axial conformation **2** to be favored over the equatorial conformation **3**. Moreover, the conformation with an equatorial aldehyde group is calculated to be more stable (ΔG = 0.83 kcal/mol).[5,6] This free energy difference corresponds to having 80% of the equatorial isomer at room temperature. The resonance integral for the interaction between the axial aldehyde group and double bond of **2** is small, whereas it is vanishingly small for **3** (see Table III).[4] According to MMP2, the supra annular effect does, in fact, exist, but it is too small to dictate the conformation such a molecule will adopt.

Kugatova-Shemyakina, Nikolaeu, and Andreev report that they obtained a 1:3 equilibrium mixture of cis–trans isomers of 6-methyl-3-cyclohexene-1-carboxaldehyde upon heating at 250 °C, and under acid catalysis at room temperature.[2,7] We calculate the free energy difference between **6** and **7** to be 0.24 kcal/mol, which corresponds to



an approximate 1:1.5 equilibrium mixture. Between the two cis conformations **4** and **5**, conformer **5** is calculated to be more stable (ΔG = 0.24 kcal/mol). Again, the nonbonded overlap in **5** lowers the energy by only a scant 1.1 calories. This energy decrease is insufficient to influence the position of equilibrium. The trans epimer **6** is more stable than its diaxial conformer **7** by about 1.1 kcal/mol. Moreover, CONII is the preferred conformation for similar reasons to those presented above.

(3) Dubois, J. E.; Fresnut, P. *Tetrahedron* **1973**, *29*, 3407.

(4) This is the π-system version of the MM2 program Allinger, N. L.; Yuh, Y. *Quantum Chem. Program Exchange* **1980**, *12*, 395. The new program is available from Molecular Design Limited, San Leandro, CA 94577, and from the Quantum Chemistry Program Exchange, Department of Chemistry, University of Indiana, Bloomington, IN 47405.

(5) Zefirov, N. S.; Chekvlaeva, V. N.; Belozerov, A. I. *Zh. Org. Khim.* **1969**, *5*, 630. These investigators examined 3-cyclohexene-1-carboxaldehyde and related systems with NMR and found that the equatorial conformer **3** was more stable than **2** (ΔG = 0.4–0.5 kcal/mol). This free energy difference indicates that the equilibrium mixture has 70% of the equatorial conformation present. These authors conclude that there is no supra annular effect as the singular form of conformational interaction.

(6) When several conformations of a single compound have similar steric energies, a Boltzmann calculation may be carried out to determine the mole fractions of each conformer present in an equilibrium mixture. Each mole fraction can then be multiplied by the respective steric energy and summed to determine the average heat content, ΔH, present in the system. The entropy of mixing may be calculated, multiplied by the temperature, and subtracted from ΔH to yield the free energy, ΔG. This is the method employed throughout this paper to calculate the free energies from steric energies. See ref 1 for more details.

(7) I. N. Nazarov, G. P. Kugatova, and V. V. Mozolis, *Zh. Obshch. Khim.* **1957**, *23*, 2635.

(8) GAUSSIAN 80 is available from the Quantum Chemistry Program Exchange, Department of Chemistry, University of Indiana, Indianapolis, IN 47405.

Table II. Distances (Å) and van der Waals Energies (kcal/mol) for CONII and CONIII 3-Cyclohexene-1-carboxaldehyde

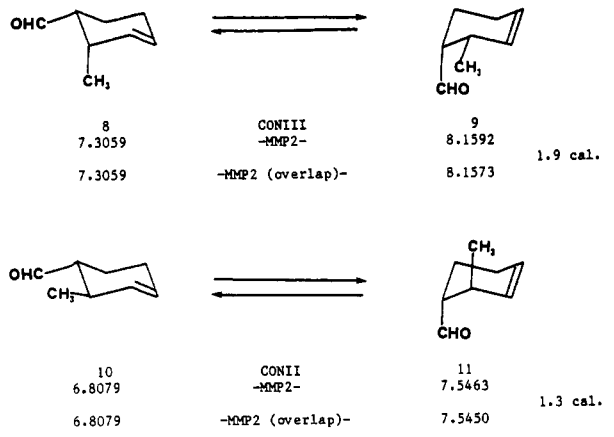
compd	conformation	distance O(17)/C-3 H(11)	energy	distance O(17)/C-5 H(14)	energy
2	II	3.915	−0.0387	3.362	−0.0623
2	III	4.863	−0.0120	2.604	0.1877

Table III. Bond Order (P) and Resonance Integral (β) for Some 3-Cyclohexene-1-carboxaldehyde Derivatives

	bond order				resonance integral			
compd	3-4	3-7	4-7	7-17	3-4	3-7	4-7	7-17
2	0.9990	0.0368	0.0394	0.8769	0.9958	0.0772	0.0974	1.0455
3	1.0000	0.0043	0.0035	0.8784	0.9965	0.0001	0.0000	0.9186
4	1.0000	0.0044	0.0036	0.8784	0.9967	0.0146	0.0058	1.0458
5	0.9990	0.0349	0.0381	0.8770	0.9959	0.0698	0.0962	1.0452
6	0.9991	0.0339	0.0364	0.8771	0.9962	0.0708	0.0909	1.0455
7	1.0000	0.0042	0.0034	0.8783	0.9981	0.0143	0.0053	1.0454
8	0.9999	0.0032	0.0026	0.8783	0.9969	0.0106	0.0048	1.0459
9	0.9980	0.0523	0.0467	0.8756	0.9963	0.1391	0.0972	1.0449
10	1.0000	0.0041	0.0034	0.8784	0.9973	0.0138	0.0057	1.0463
11	0.9990	0.0364	0.0390	0.8770	0.9961	0.0763	0.0961	1.0455

Obviously, the small energy difference between *cis*-6-methyl-3-cyclohexene-1-carboxaldehyde conformations **4** and **5** implies that there is not much of a difference in the A values between $-CH_3$ and $-CHO$.[9] Furthermore, the free energy difference corresponding to the 1:3 equilibrium mixture of 6-methyl-3-cyclohexene-1-carboxaldehyde corresponds to the energy difference between cis aldehydes **4** and **5** and principally the trans diequatorial **6**.

For 2-methyl-3-cyclohexene-1-carboxaldehyde, our calculations indicate similar results. The computed supra annular stabilization only amounts to 1.3–1.9 cal (see Table IV). The overlap, as before, is essentially nonexistent for **8** and **10**.



The most stable cis conformation has the carboxaldehyde group equatorial and the C-2 methyl axial. The energy difference between **8** and **9** can be traced to bending and van der Waals effects. No doubt, allylic strain tends to favor conformer **8** over **9**, as the former has the allylic methyl group axial. The trans epimer preferentially exists as conformer **10** rather than **11**, where the ring substituents are equatorial and no supra annular interactions occur. The principal component in the energy difference between **10** and **11** has its origins with torsional strain (gauche interactions).

GAUSSIAN 80 **Examination.** Examining the axial–equatorial equilibrium for 1-carboxaldehyde-3-cyclohexene systems with ab initio methods provided findings identical with MMP2 results. Taking the MM2 minimized coordinates for equatorial compound *3* (CONII) and its axial epimer **2** (CONII) and employing them in Pople's GAUSSIAN 80 program,[8] we performed several single-point Hartree–Fock calculations using 6-21G and STO-3G* basis sets.

For the 6-21G basis set, GAUSSIAN 80 has the equatorial isomer as the more stable by 1.07 kcal, which is in agree-

Table IV. Final Steric Energies (kcal/mol) for Various 3-Cyclohexene-1-carboxaldehyde Derivatives

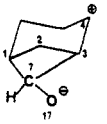
	CONI	CONII	CONIII
3-Cyclohexene-1-carboxaldehyde (AX) (**2**)			
MM2	6.8228	5.7901	6.0592
MMP2	6.8099	5.7739	6.0439
MMP2 (overlap)	6.8097	5.7726	6.0421
3-Cyclohexene-1-carboxaldehyde (EQ) (**3**)			
MM2		5.0568	5.0591
MMP2	6.0095	5.0411	5.0427
MMP2 (overlap)	6.0095	5.0410	5.0427
cis-2-Methyl-3-cyclohexene-1-carboxaldehyde (AX) (**9**)			
MM2	8.2462	8.2817	8.1733
MMP2	8.2267	8.2730	8.1592
MMP2 (overlap)	8.2265	8.2720	8.1573
cis-2-Methyl-3-cyclohexene-1-carboxaldehyde (EQ) (**8**)			
MM2	8.6694	8.2081	7.3222
MMP2			7.3059
MMP2 (overlap)			7.3059
trans-2-Methyl-3-cyclohexene-1-carboxaldehyde (AX) (**11**)			
MM2	8.4558	7.5637	7.7729
MMP2		7.5463	
MMP2 (overlap)		7.5450	
trans-2-Methyl-3-cyclohexene-1-carboxaldehyde (EQ) (**10**)			
MM2	7.2773	6.8226	6.9682
MMP2		6.8079	
MMP2 (overlap)		6.8079	
cis-6-Methyl-3-cyclohexene-1-carboxaldehyde (AX) (**5**)			
MM2	7.5530	7.0466	7.5157
MMP2		7.0296	
MMP2 (overlap)		7.0285	
cis-6-Methyl-3-cyclohexene-1-carboxaldehyde (EQ) (**4**)			
MM2	8.6294	7.1989	7.6038
MMP2		7.1833	
MMP2 (overlap)		7.1833	
trans-6-Methyl-3-cyclohexene-1-carboxyaldehyde (AX) (**7**)			
MM2	8.6289	7.7058	7.9928
MMP2		7.6888	
MMP2 (overlap)		7.6878	
trans-6-Methyl-3-cyclohexene-1-carboxaldehyde (EQ) (**6**)			
MM2	6.9189	6.6685	7.1304
MMP2		6.6527	
MMP2 (overlap)		6.6527	

(9) The *A*-values are given by Hirsch (Hirsch, J. A. In "Topics in Stereochemistry"; Allinger, N. L., Eliel, E. L., Eds.; Wiley: New York, 1967, Vol. I) as respectively 1.70 and 1.30 (where the latter is not reported but is estimated by us from the values of the $-COCH_3$ (1.27) and $-CH{=}CH_2$ (1.35 groups).

Table V. Calculated ab Initio Electronic Charges for CONII Axial and Equatorial 3-Cyclohexene-1-carboxaldehydes 2 and 3

atom	atom	electronic charges	
		equatorial	axial
3	C (sp^2)	6.20771	6.19140
4	C (sp^2)	6.20296	6.20051
7	C (sp^2)	5.60850	5.60905
17	O	8.51863	8.51290

ment with MM2. Moreover, if there were a substantial overlap between the aldehyde and double bond moieties, contributing to a reduction in energy, we would expect to find this reflected in the gross orbital charges for the charge on the pertinent atoms. Specifically, resonance as shown should lead to a decrease in the electronic charge on atom 4, and an increase on atom 17 (oxygen) in the axial conformation. As indicated in Table V, the charges are essentially the same for the axial and equatorial isomers. Carbon 4 does lose a small amount of charge, but so does oxygen.



Conclusion

MMP2 force field calculations have been carried out on several carbonyl-substituted 3-cyclohexene compounds in order to determine the quantitative results for the proposed supra annular effect. When nonbonded overlap between the alkene and axial carbonyl carbons was included in the SCF routine of the MMP2 energy minimization process, we obtained a small energy reduction of 1-2 cal/mol. When this overlap was included for the corresponding equatorial isomer, there was no energy change. Clearly, this small energy stabilization is too small to force the aldehyde substituent to adopt an axial conformation and influence the equilibrium position. GAUSSIAN 80 calculations confirm our MMP2 results. Moreover, our studies indicate that there is a preference for the aldehyde group to be equatorial, not axial. One might try to explain the reduction of reactivity for the alkene and carbonyl functionalities in various ways. However, we conclude that any explanation which requires a supra annular resonance effect to yield a ground state conformational change cannot be correct.

Acknowledgment. We thank the National Institutes of Health for their support (5 R24 RR02165).

Registry No. 2, 100-50-5; 5, 36635-34-4; 7, 36635-33-3; 9, 873-30-3; 11, 766-48-3.

Nucleophilic Catalysis of Hydrolysis of a Schiff Base by Amines. Intramolecular Catalysis of Transimination

Tadashi Okuyama,* Hiroaki Nagamatsu, Masakazu Kitano, and Takayuki Fueno

Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

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Certain groups of amines were found to catalyze hydrolysis of *N*-(*o*-methoxybenzylidene)-2-methoxyethylamine through transimination. The rate-determining transimination was followed by rapid hydrolysis of an intermediate Schiff base. Rate constants for the transimination with simple, but less basic, amines change sigmoidally with pH and are buffer-dependent in accord with a mechanism involving a trapping of the incipient tetrahedral intermediate T_1^+ by a proton transfer to acids or bases. Morpholine behaved similarly. In the reaction with bifunctional amines carrying an internal amino group, rates are independent of both pH and buffer concentrations. Initial nucleophilic attack of these amines is rate determining in the whole pH range examined because of the rapid trapping of T_1^+ by an intramolecular proton transfer.

Schiff bases are important intermediates involved in many enzymic transformations.[1] Mechanisms of formation and hydrolysis of these compounds have been investigated in great detail and are now understood very well.[2] Transimination (interconversion among Schiff bases) is also a key step of biological reactions involving, e.g., pyridoxal 5′-phosphate dependent enzymes.[3] However, because of the close similarities of the absorption spectra of the reactants and products of this reaction, investigations using model systems pertinent to the enzyme seem to be limited.[3,4] Nucleophilic catalysis of Schiff base formation of anilines[5] and secondary amines[6] is known as a typical example of such catalysis and involves transimination as a rapid process.[7]

The hydrolysis must also be facilitated by certain amines as a microscopic reverse of the formation but no examples of the catalysis have so far been reported. Such catalysis is effective only when the transimination is rapid and the intermediate Schiff base is more readily hydrolyzed than the starting substrate. Since the hydrolysis of Schiff base was found to be catalyzed by an internal general base[8-10] and the transimination may also be facilitated by the internal base, amines carrying an appropriate base group can

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