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Calculated Chemical Shifts as a Fine Tool of Conformational Analysis: An Unambiguous Solution for Haouamine Alkaloids

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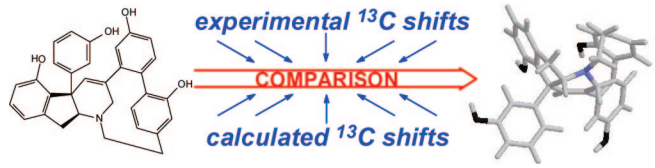
Received December 30, 2007



In this study, for the first time, conformational analysis by calculated chemical shifts (CCS) deals with a real conformational problem of a large biomolecule. This new methodology is applied to haouamine A, which is much more stereodynamically puzzled than the small models used to validate previous CCS-based conformational studies. Thorough NMR experiments by Zubía et al. on this exotic polyfunctional paracyclophane alkaloid could not determine which experimentally detected interconversion of this compound occurs in solution, rotation or N-inversion. The present study uses CCS to locate the lowest energy conformers and thus to identify the observed stereodynamic process. Molecular mechanics calculations were used to explore the conformational space of this polycyclic system, and then the geometry of located conformers was refined by ab initio calculations at the B3LYP/6-31G(d,p) level; an implicit model for acetone solution was employed. Calculated relative energies are considered too inaccurate to identify the lowest energy (i.e., those detectable by NMR) conformers. Instead, rational regression analysis of CCS for carbon atoms using B3LYP/6-31+G(d)//GIAO-based calculations singled out two conformers from a large set of alternative low energy structures, although solvation shell was not explicitly included in the model. For only these two conformers, the differences in CCS ($\Delta\delta$) for selected pairs of carbons are very similar to the experimental $\Delta\delta$ values. Thus, the conformers monitored by NMR have now been identified; their piperideine ring is of ^{1}Sf and Sf_1 (sofa-shaped) geometry. This azacycle appears to be flexible despite the presence of the ethylenebiphenylene bridge in haouamines. Interconversion between the conformers probably occurs via a concerted process of inversion of the piperideine ring, N-inversion coupled with rotation around the C−N bond, and rotation around two C−C bonds in the ethylenebiphenylene bridge. This CCS method of conformational analysis is sufficiently simple and reliable that if chemical shifts for a pair of the same carbons are sufficiently different in routine ^{13}C NMR spectra of stereoisomers (ca. ≥2 ppm), the "resolving power" of the CCS technique may rival that of NMR techniques.

Introduction

Chemical and stereochemical structure was recently determined by complex 2D and 3D NMR experiments, as well as X-ray diffraction analysis, for two new related marine alkaloids, one of which is haouamine A (**1**; Figure 1).[1] However, both compounds possess a labile molecular structure in solution.[1] At ambient temperature each of these unusual azaparacyclophane alkaloids exists as two NMR-detectable interconverting forms (considered as isomers[1,2]) having the same backbone and the same stereochemistry of the ring fusion, whereas crystallization of **1** afforded a pattern of a single molecular 3D geometry (structure *C1* in Figure 2). Redissolving this substance resulted in the primary mixture of the two equilibrating molecular forms.[1] Routine integration of the ^{1}H signal intensities gave their ratio (e.g., 2:1 in deuteroacetone).

The molecular geometry of these forms of **1** in solution and hence the detected molecular transformation are still a chal-

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10.1021/jo702766x CCC: $40.75 © 2008 American Chemical Society
Published on Web 07/03/2008

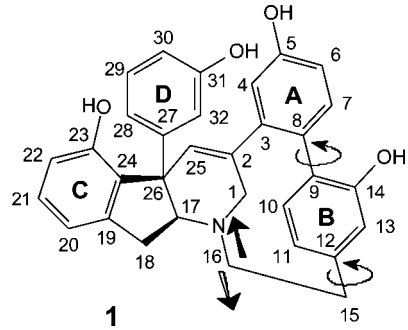

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FIGURE 1. Haouamine A and the intramolecular motions (N-inversion and phenylene group rotation) considered in refs 1 and 2.

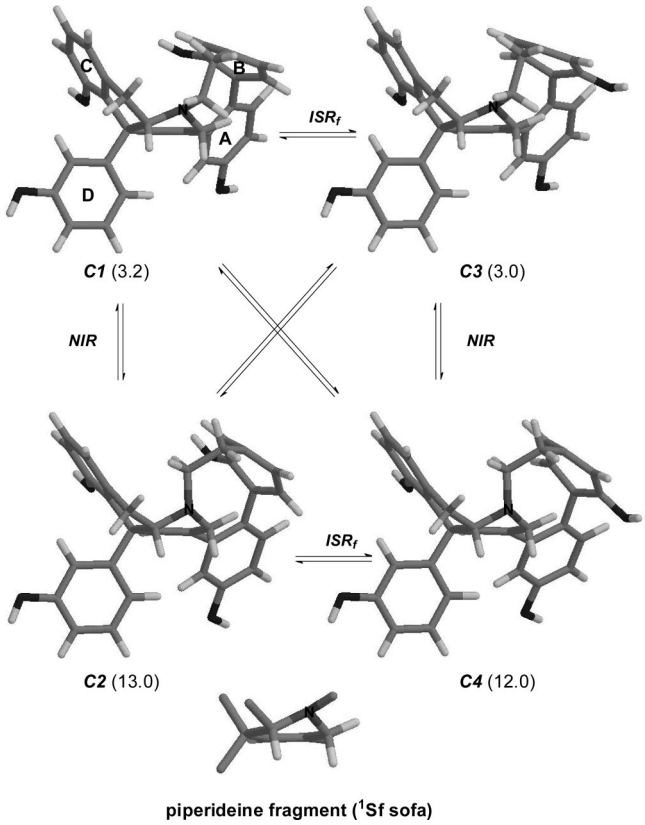

FIGURE 2. Simplified scheme of interconversion of conformers *C1*−*C4* of amine **1**. The geometries shown have been optimized at the B3LYP/6-31G(d,p) level (this work); see ref 3 for explanation of formal isolated rotation (ISR_f). Values of ΔE [kcal/mol; relative to conformer *C12* (see below)] are calculated for acetone solution and are shown in parentheses.

lenging conformational problem. It was carefully concluded[1] that structure *C1* corresponds to a favored form (depicted *M1* here) detected in deuteroacetone solution.[3] However, the conformation of some fragments (the piperideine cycle,[3] as well as rotational orientations of ring D and O−H bonds in the OH groups) in *M1* are outside the scope of this thorough NMR study. The minor form (*M2*) possesses the same stereochemistry of the ring fusion;[1] nonetheless, the general conformational motif in *M2* remained obscure. Rotation of ring B (see ref 4) and inversion of the piperideine nitrogen in *C1* were discussed as possible alternatives for the dynamic process observed by NMR, and the spatial structure of *M2* was proposed as resulting either from N-inversion (i.e., corresponding to *C2*; see ref 4) or from the *p*-phenylene rotation (i.e., corresponding to *C3*; ref 4). Additional NMR experiments with modified alkaloids[1] were unable to resolve among the alternatives.

The interconversion hypothesis[1,2] restricts itself to consideration of only rotamers (*C1* vs *C3*)[4] and N-invertomers (*C1* vs *C2*)[4] of amine **1**. It is controversial whether the 3-piperideine ring in **1** is rigid; partially saturated six-membered cycles,[5a] including 3-piperideines,[5b] are flexible. There is no reason to assume that the ethylenebiphenylene bridge of haouamines locks the tetrahydropyridine ring into the ^{1}Sf sofa conformation (Figure 2). Rather, other conformations of this azacycle could not be ignored.

Interconversion *M1* ⇌ *M2* is slow on the NMR time scale.[1] Unfortunately, studies on the rates of hindered conformational dynamics in alkyl amines[5b,6a–c] (including N-fused bicycles[6d]) and paracyclophanes[7a–c] are not relevant to analysis of amine **1**: because of a covalent junction of two conformationally mobile fragments (the tetrahydropyridine cycle and the ethylenebiphenylene chain), the system cannot be formally split into two stereodynamically independent fragments. Formally, there are three types of intramolecular motions for this system, isolated rotation (ISR_f) of the *p*-phenylene ring,[4] nitrogen inversion−rotation (NIR) of the N-alkyl substituent,[8a,b] and tetrahydropyridine ring inversion (RI). As a result of the configurational instability of the bridgehead pyramidal nitrogen, these motions may be coupled, and no estimates are available for the kinetics of these concerted stereodynamics. Besides, both *p*-phenylene rings are nonplanar,[1] and hence, the tetrahydropyridine−biphenylene fragment of **1** is strained. Therefore no intramolecular motion in **1** could be characterized by energetics of the similar motion in minimal structural analogs; such a system is actually a new stereodynamic puzzle.

(3) In the original text: ".isomer I could have a stereochemistry as represented in Figure." This accurate NMR study[1] reasonably doesn't claim that the obtained ROESY correlations indicate a sole geometry of the tetrahydropyridine ring of *M1* in solution. Indeed, these experimental NOE-based data are compatible with a ^{1}Sf and Sf_1 sofa as well as a $^1H_6$ half-chair conformation (see Supporting Information).

(4) Concerning rotation, it is discussed[1] as "atropoisomerism of the 3-aza-[7]-paracyclophane system". This description of rotation may be interpreted only as rotation of ring B. Geometries, which result from the proposed rotation or N-inversion in *C1*, are not specified in ref 1. The only source of structures *C3* or *C2*/*C4* are the present calculations. On the other hand, formally, these structures may be obtained via rotation of ring B or N-inversion, respectively (Figure 2; see Figure 3 for similar structures). No other conformers, which could appear as a result of these intramolecular motions, e.g., additional ring B rotamers, have been located (this work). Thus, conformers *C2*, *C3*, *C4*, etc. correspond to general structures implied in ref 1. In fact, rotation of ring B is sterically hindered (see Supporting Information for details). However, consideration of interconversions *C1* ⇌ *C3*, *C5* ⇌ *C7*, etc. cannot be withdrawn. For instance, additional calculations show that this ring rotation might result from a sequence of intramolecular chemical transformations which provide interconversion *C1* ⇌ *C3* (see Supporting Information). Other, unexpected mechanisms couldn't be excluded. Thus, ISR_f in Figures 2 and 3 means formal rotation of phenolic ring B.

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The initial interconversion hypothesis also does not take into account ionized forms, namely, species with protonated amino and deprotonated phenol moieties. The skeleton of these alkaloids brings the amino and phenolic moieties groups quite close together, and formation of betains NH^+-O^- due to intramolecular proton transfer cannot be excluded a priori. It is known that mixtures of aliphatic amines (weak bases) and phenols (weak acids) exist in solution as multiple complexes in equilibrium.[9a–e] Hence, the problem of the molecular structure of haouamines in solution and their structural dynamics is actually more complicated than can be analyzed with the starting interconversion hypothesis.[1]

It is hardly surprising, then, that a tenacious NMR study[1] could not solve the conformational problem for haouamine alkaloids. That study utilized a common conformational model restricting itself by consideration of only ISR and N-inversion. Herein, in continuation of structural studies[1] of **1**, this polycycle is approached without any preliminary suggestion regarding possible structures of conformers, as well as possible intramolecular stereomutations.

Calculated chemical shifts (CCS) are the key tool that was used to solve the problem of haouamine alkaloids. The principle of this methodology for structure elucidation[10a] is extremely simple. Quantum mechanics ab initio calculations are capable of supplying sufficiently accurate values of NMR chemical shifts for different nuclei, e.g., for carbons. Comparison of experimental ^{13}C chemical shifts and the corresponding CCS for alternative hypothetical structures makes it possible to identify the true structure(s). Here we have employed computational conformational analysis, as well as calculations of ^{13}C chemical shifts, in order to reveal which molecular forms are involved in the poorly understood intramolecular transformations of haouamine alkaloids.

Results and Discussion

Computational conformational analysis was performed in order to locate the most stable conformers of polycycle **1**, as well as its betainic structures NH^+-O^-, or in other words the molecular forms of lowest energy that have been characterized[1] by individual NMR spectra. Relative stability of the structures identified was estimated by calculating the molecular energy difference ΔE for conformers of this amino polyphenol with (a) free, nonionized functional groups (the tertiary amino R_3N and phenolic OH moieties) and (b) charged HN^+R_3 and ArO^- groups.

1. Energy Calculations. Initially, conformations of **1** were generated via a random conformational search followed by geometry optimization using the MM3* force field (see Supporting Information and Experimental Section for details). All structures, ionic and nonionic, were reoptimized using ab initio calculations that included an implicit approximation for acetone solution[10b] (see Experimental Section). The geometry of more stable conformers was refined at the B3LYP/6-31G(d,p) level, and the ^{13}C chemical shifts of those structures with $\Delta E \leq 4$ kcal/mol relative to the B3LYP/6-31G(d,p)-located global minimum were calculated at the B3LYP/6-31+G(d) level using the GIAO methodology[11a–c] (see Experimental Section and Supporting Information).

a. Conformers with Free Amino and Phenol Groups. In calculations involving the implicit model of acetone environment, the B3LYP/6-31G(d) "razor" cuts off high energy structures, leaving a compact group of more stable conformers of polycycle **1**: they lie within a ~5.2 kcal/mol ΔE range over the lowest energy conformer. There is a gap between the low and high energy conformers. The latter occupy conformational space starting at $\Delta E = 12.0$ kcal/mol. Structure refinement at the B3LYP/6-31G(d,p) level was performed for all low-energy conformers and the four lowest-energy structures from the high energy group (C2, C4, C6, and C8). After refinement, the energy gap between the two groups of conformers remained the same, and the low-energy conformers were concentrated in the 0−4.5 kcal/mol interval of ΔE. This significant difference in stability between the two groups allows the conformations at higher energy to be rejected with confidence.

The piperideine cycle in all of the conformers adopts exclusively a sofa shape. Moreover, there are only two sofa conformations, slightly distorted ^1Sf and Sf_1 sofas, i.e., flattened rings with the out-plane N atom (Figures 2 and 3). Those conformers bearing an equatorial exocyclic N-substituent in the tetrahydropyridine ring form the group of low energy conformers, e.g., *C1* and *C3* of the ^1Sf-family (Figure 2), as well as *C5* and *C7* of the Sf_1-family (Figure 3). Sofas with an axial N-substituent, e.g., *C2*, *C4*, *C6*, and *C8*, belong to the group of high energy conformers. Thus, only equatorial conformers made it to the next stage of analysis. Nevertheless, already after the first stage of the conformation consideration, one may conclude that polycycle **1** is more flexible than previously thought and, hence, the original stereodynamics hypothesis[1,2] is inadequate.

Some of the low-energy conformers share the same geometry of the pentacyclic backbone but differ solely by (a) rotational orientation of phenyl ring D or (b) opposite orientation of phenolic O−H bonds (rotational orientation around C−O bonds). For instance, conformers *C9* and *C10* result from the Ph rotation by ~180° in *C1* and *C7*, respectively Figure 4).

Accordingly to the energy calculations, rotamers that have an intramolecular H−O···H−O bond due to an "in"-orientation of the phenolic moiety of the ring A (e.g., *C5*, *C6*, *C12*, and *C16*) are more stable than the corresponding "out"-rotamers. The *C12* sofa, with intramolecular H-bonding, is the structure of minimal calculated energy. However, because of the inaccuracy in energy calculations (see Supporting Information), *C12* cannot be considered as the lowest energy conformer on the basis of only ΔE values. The ΔE values for pairs of 32 studied C−O rotamers (e.g., pairs *C9* and *C11*, *C5* and *C12*, *C1* and *C13*, *C7* and *C14*, *C10* and *C15*, or *C5* and *C16* and others not shown) do not differ more than 1.5 kcal/mol between the pair members, preventing any conclusion from being drawn about the relative stability of the C−O rotamers of a pair. Also concerning other conformers, since almost all of the low energy structures fall below the 4 kcal/mol threshold of ΔE accuracy

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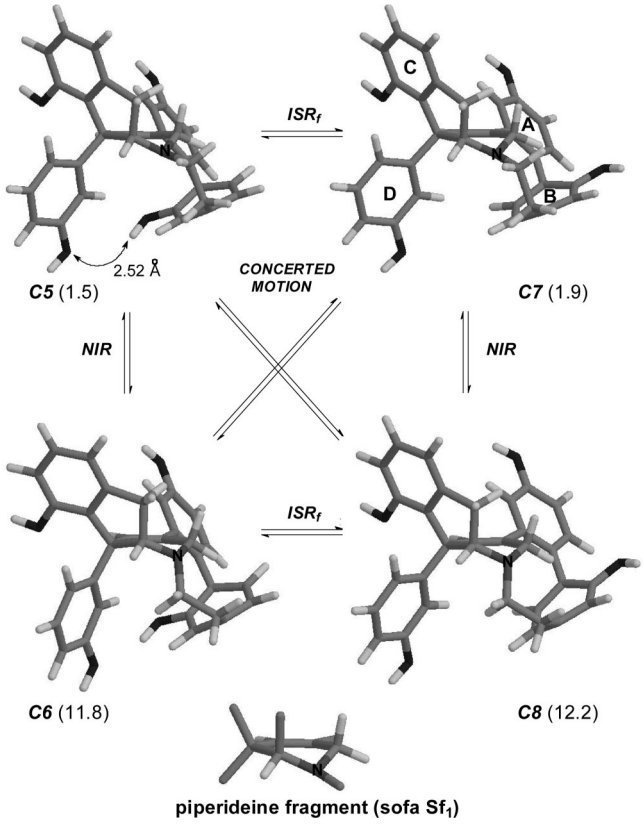

FIGURE 3. Simplified scheme of interconversion of conformers *C5*−*C8* (Sf_1 sofa conformers). The structures optimized at the B3LYP/6-31G(d,p) level, and the corresponding ΔE values for acetone solution are shown in parentheses (see ref 3 for explanation of ISR_f). ΔE values are given in kcal/mol relative to the energy of *C12*, the conformer of the lowest calculated energy. Intramolecular H-bondng in *C5* and *C6* is indicated by arrows.

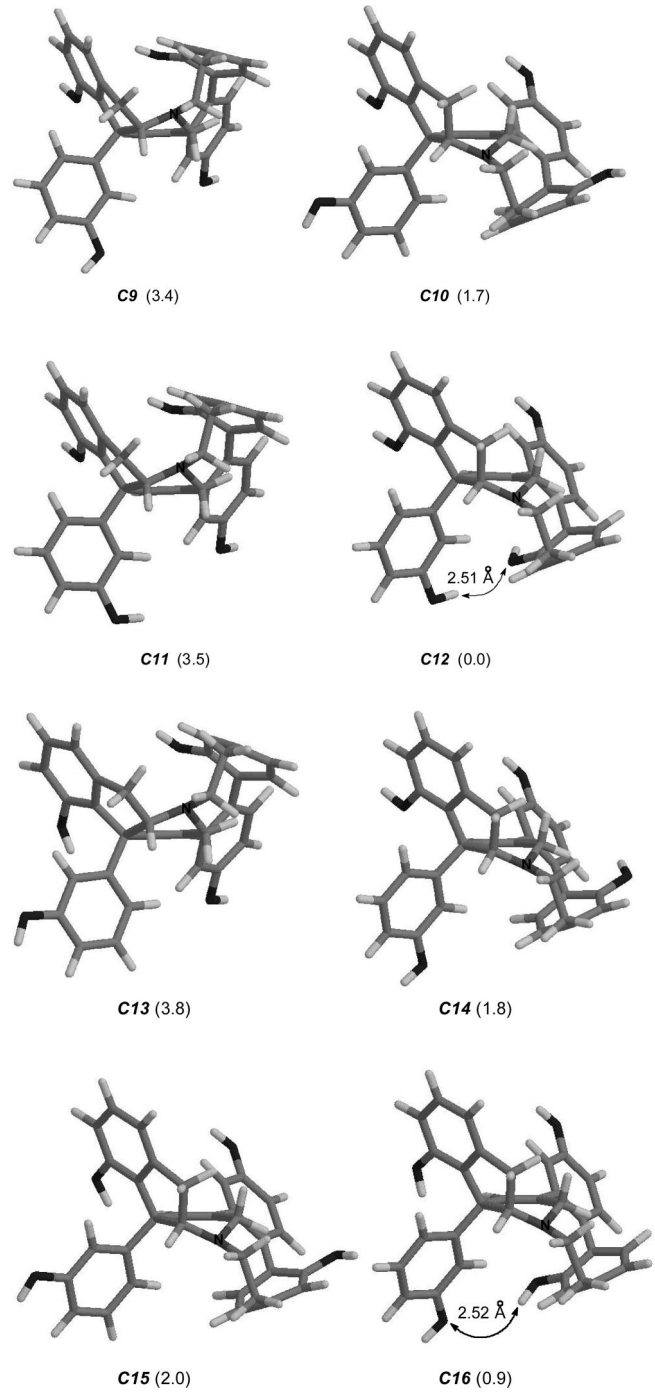

FIGURE 4. Structures of conformers *C9*−*C16* optimized at the B3LYP/6-31G(d,p) level. Relative energies in kcal/mol, calculated for acetone solution and expressed relative to the energy of *C12*, are shown in parentheses. Arrows indicate intramolecular H-bonding.

(see above and Supporting Information), additional data are needed in order to rank them in order of stability.

b. Structures with Ionized Amino and Phenol Groups. Calculations at the B3LYP/6-31G(d) level for acetone solution suggest that proton transfer from any of the phenol functions in tetraphenol **1** to the amino nitrogen lead to betains of high energy, with ΔE of more than 8 kcal/mol relative to the lowest energy minimum *C12*. Only intramolecularly H-bonded structures, which have a short O−H···O$^+$ bond of 1.68 Å (e.g., *C17*; Figure 5), are near the *C12* minimum (ΔE = 2.9 kcal/mol relative to *C17*). Conformers with an opposite orientation of the O−H bond in rings A, B, or C are not shown; their energy is ∼0.5−1.0 kcal/mol higher (again, relative to *C17*). However, energy reminimization at the B3LYP/6-31G(d,p) level yielded a ΔE of 4.7 kcal/mol for *C17*; ΔE between *C12* and other high energy betain structures increased to 8.8 kcal/mol. Taking into account their error margin, one may conclude that based on *energy calculations, C17* and related C-OH rotamers are capable of appreciable contribution to the NMR spectra[1] of the minor component *M2*. In other words, these calculations do not reveal whether *M2* is mostly a nonionized or ionized form of **1** or an equilibrating mixture of these considerably populated forms. Other (high energy) betains are not considered further.

2. Conformational Analysis via CCS. CCS are supplied by different levels of ab initio theory with different accuracies.[12a–k] For unsubstituted tertiary cyclic alkylamines, accurate predictions of experimental ^{13}C chemical shifts have been provided[13] by the GIAO methodology of absolute shieldings[10b,11a–c] accompanied by the B3LYP hybrid functional calculations using only the 3-21G basis set. A more extended basis set (B3LYP/6-31G(d)//GIAO calculations) has proven accurate for predicting ^{13}C shifts of oxymorphone, a polyfunctional tertiary amine.[14]

Since the pioneering introduction of CCS to conformational analysis,[12e] they have been used more to examine this methodology than for practical applications. The example of mono- and polycyclic models below illustrates the limitations on system size and solvation with conventional CCS methodology. It has been able to distinguish between the equatorial and axial conformers of methylcyclohexane,[12e] as well as between the

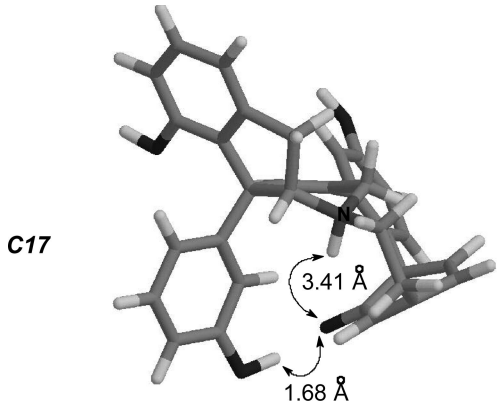

FIGURE 5. Betain structure *C17* optimized at the B3LYP/6-31G(d,p) level. Arrows indicate interatomic distances.

endo and exo conformers of 2-methyl-2-azabicyclo[2.2.1]-heptane in nonpolar solvents, approximating the medium by vacuum conditions. However, modeling these conditions, it has not been possible to reveal unambiguously a chair or twist geometry of ring A of a steroid molecule in chloroform[12e] (see Supporting Information for notes related to the accuracy of CCS).

Linear regression removes the systematic error from calculated absolute isotropic shieldings and the improved values (predicted chemical shifts) have been shown[12c–e,j,13] to be near experimental chemical shifts. Herein, CCS, which are evaluated directly as the difference between these calculated shieldings for the carbon atoms of the conformers under analysis and for tetramethylsilane (TMS),[12a,f,k,l] have been used. Instead of calculated absolute shieldings, CCS themselves have been subjected to regression analysis. Indeed, linear correlation of CCS versus experimental shifts and linear correlation of calculated shieldings versus experimental shifts are of the same accuracy (see Supporting Information). However, as analogs of experimental chemical shifts (i.e., of similar relative values), TMS-derived CCS values are more convenient.

a. Comparison of δ Values. Experimental ^{13}C shifts (δ) for M1 and M2 in acetone[1] were compared with CCS of low energy conformers C_i of **1**. The sp^3-hybridized carbons were chosen excluding the 18-positioned carbon because its experimental shift value in *M2* is unavailable. Aromatic carbons were not taken into consideration (see Supporting Information for the explanation); regression analysis for experimental (δ_{exp}) and calculated (δ_{calc}) values was performed using standard error of regression (σ_r) as a selection criterion. Table 1 shows representative examples of CCS; other rotamers showed lower correlations and so were not included in the table.

TABLE 1. Experimental[1] and Calculated ^{13}C NMR Chemical Shifts (δ, ppm)[a] for Aliphatic Carbons of Alkaloid 1 and the Corresponding Standard Error of Regression σ_r (ppm)

entry	C-1	C-15	C-16	C-17	C-26	σ_r for*M1*	σ_r for*M2*
M1[b]	53.0	40.0	56.0	74.7	62.5		
M2[b]	44.2	38.1	56.6	73.1	58.4		
C1	54.2	43.3	58.6	77.8	66.2	1.1	3.4
C3	53.9	42.6	58.7	77.1	68.9	2.3	4.2
C5	47.0	42.3	59.7	76.8	60.7	4.6	0.9
C7	45.6	42.7	60.2	75.5	64.8	5.3	2.2
C9	54.4	43.8	59.8	79.4	68.8	1.7	3.6
C10	43.8	40.2	57.9	74.8	63.5	5.2	2.2
C11	55.0	43.7	59.3	78.5	69.1	1.8	4.0
C12	46.8	42.5	59.4	76.2	60.9	4.5	0.8
C13	52.7	43.8	60.1	76.6	64.9	2.0	1.9
C14	46.4	42.7	59.7	75.1	65.0	4.8	2.2
C15	42.5	41.5	58.9	77.5	61.8	6.3	2.4
C16	46.1	41.8	60.4	77.2	59.6	5.3	1.5
C17	51.9	38.2	59.9	78.2	63.9	2.0	3.1

[a] CCS [at the B3LYP/6-31+G(d) level] for the TMS carbons is 190.9 ppm. [b] Experimental δ values (δ_{exp}).

Clearly, the calculation accuracy is sufficiently high for the selected carbons. The set of structures *C1*, *C3*, *C9*, *C11*, *C13*, and *C17* and the set of structures *C5*, *C7*, *C10*, *C12*, *C13*, *C14*, *C15*, and *C16* were found to be related, respectively, to *M1* and *M2*; they are characterized by $\sigma_r \leq 2.5$ ppm. High σ_r values (more than 3 ppm) reliably exclude conformers *C5*, *C7*, *C10*, *C12*, *C14*, *C15*, and *C16* from being related to *M1*, and structures *C1*, *C3*, *C9*, *C11*, *C13*, and *C17* from being related to *M2*. Lower values of σ_r are of the magnitude of the δ calculation error and are therefore insufficient to identify the lowest energy conformers among the low σ_r structures. Although this initial selection decreased the number of structures to be considered, all of the structures *C1*−*C17* were re-examined by an additional criterion.

b. Comparison of $\Delta\delta$ Values. Absolute calculation error is significant (up to 5 ppm) for the most aromatic carbons of **1** for conformers resulting from the initial selection (*C1*, *C3*, *C9*, *C11*, *C13*, and *C17* vs *M1,* as well as *C5*, *C7*, *C10*, *C12*, *C13*, *C14*, *C15*, and *C16* vs *M2*; see Supporting Information). However, the accuracy for the difference in chemical shift for two atoms ($\Delta\delta$) should be higher as a result of the subtraction of calculation errors of close values (see Supporting Information).

Chemical shifts of some carbons are appreciably different for *M1* and *M2*; in other words, they are "conformation-sensitive," e.g., C-1 and C-11. Therefore calculated $\Delta\delta$ for a pair of aliphatic or aromatic carbons from one domain of the same conformer (e.g., for the C-11/C-13 pair) were chosen for comparison with the experimental $\Delta\delta_{exp}$ for these atoms. For each pair at least one of the carbons shows the conformation-sensitive chemical shift. Thus, the set of selected $\Delta\delta_{calc}$ for these pairs for a conformer is representative characteristics of this conformer in terms of CCS. Then these sets of $\Delta\delta_{calc}$ values for the conformers under study were compared with the corresponding sets of $\Delta\delta_{exp}$ values. As with the regression analysis of δ values, the standard error of regression for $\Delta\delta_{exp}$ versus $\Delta\delta_{calc}$ was used to assess the correlation quality (see Table 2).

The regression error σ_r clearly singles out conformers *C1*, *C9*, and *C11* as corresponding to *M1* and conformers *C7*, *C10*, and *C15* as corresponding to *M2*. Values of σ_r are low for these conformers and, more importantly, are significantly less (by at least 3 ppm) than the values for all other structures. This $\Delta\delta$-

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TABLE 2. Experimental[1] ($\Delta\delta_{exp}$), Calculated ($\Delta\delta_{calc}$), and Standard Regression Error Values (σ_r) for differences of ^{13}C NMR Chemical Shifts ($\Delta\delta$, ppm) for Selected Carbon Pairs of Conformers of Alkaloid 1

entry	Δδ C-16/C-1	Δδ C-12/C-9	Δδ C-11/C-13	Δδ C-26/C-16	Δδ C-27/C-32	σ_r for *M1*	σ_r for *M2*
M1[a]	3.0	16.4	−4.0	6.5	32.7		
M2[a]	12.4	19.4	12.9	1.8	38.5		
C1	4.4	17.1	−4.7	7.6	36.4	0.9	9.8
C3	4.8	18.9	17.4	10.2	35.3	7.9	6.2
C5	12.7	11.6	−0.2	1.0	40.3	8.1	7.1
C7	14.6	17.4	15.9	4.6	37.2	8.5	1.8
C9	5.0	16.5	−4.4	9.2	32.3	1.6	9.8
C10	14.1	18.6	17.4	5.6	42.0	9.7	2.5
C11	4.3	16.8	−3.9	9.8	30.5	1.8	9.7
C12	12.6	16.6	−1.1	1.5	37.4	6.2	6.8
C13	12.2	14.8	−3.7	4.8	37.4	5.4	8.8
C14	18.6	22.6	12.2	5.3	35.9	7.2	3.1
C15	19.3	22.2	16.2	2.9	38.9	9.7	2.8
C16	13.4	12.5	−0.1	−0.9	40.4	8.7	6.7
C17	12.0	−1.1	15.2	4.0	29.4	12.1	9.5

[a] Experimental Δδ values ($\Delta\delta_{exp}$).

based identification of conformers is in full agreement with the initial, wider selection based on δ. Analysis of CCS has proved that betainic structures are not involved in the NMR-observed[1] equilibrium: a single low energy betain *C17* (Section 1b) shows no acceptable correlation with either *M1* or *M2*. Thus, the detected interconversion in **1** is purely conformational.

Remarkably, *C1*, *C9*, and *C11* (group i) share the same geometry of the flexible tetrahydropyridine−ethylenebiphenylene fragment, with the azacycle adopting the ^{1}Sf geometry, whereas *C7*, *C10,* and *C15* (group ii) show the alternative geometry of this fragment, with an Sf_1-shaped tetrahydropyridine ring. Ring B is of an "in"-orientation of the 14-positioned OH for group i. It is turned by ∼180°, orienting this hydroxyl "out" for group ii. Within each group, the structures are rotamers related by rotation of the Ph ring D or by formal rotation around C−O bonds. Thus, among the low σ_r conformers, regression analysis has clearly separated two certain geometries of the tetrahydropyridine−ethylenebiphynelene backbone. Group i does not contain 3D structures of the group ii geometry of this fragment, and vice versa, group ii does not contain conformers of this geometry that is related to group i. This means that conformational problem of polycycle **1** is actually solved in general. The observed[1] conformational transformation is due to the flexibility of the tetrahydropyridine−ethylenebiphynelene fragment and the interconversion occurs between its two geometries; these geometries are shown in Figure 6 for the example of conformers *C1* and *C7*.

The presence of rotamers in each of the two groups is not surprising, since calculated chemical shifts are similar for some rotamers because of their closely related 3D geometry. *C1* and *C13*, for example, differ only by an opposite orientation of the O-H bond in the plane of ring C. Rotation of phenyl groups as well as rotation around C-OH bonds are usually fast in the NMR time scale;[15a–c,16a,b] therefore experimental NMR spectra of *M1* and *M2*[1] are a superposition of spectra of individual Ph rotamers and C-OH rotamers. If those are of comparable stability, the resulting experimental spectra do not correspond to the spectrum of any individual rotamer. Although values of σ_r for *C1* or *C7* are the lowest values in their conformer groups, standard deviations σ_r for rotamers *C9* and *C11* or *C10* and *C15* are relatively close to these low σ_r values (Table 2). Therefore, at this stage, we leave open the question of whether the lowest value of σ_r for conformer *C1* indicates its total predominance over *C9* and *C11* in major form *M1*. Values of σ_r for *C9* and *C11* are higher by only 0.7 and 0.9 ppm, respectively, indicating that these rotamers may appreciably contribute to the equilibrium. Similarly, no definitive conclusion can be made regarding the ratio of *C7* to *C10* and *C15* in minor form *M2*. These data make it possible to say only that conformer *C1*, which is the form of **1** in crystals,[1] is also favored in acetone solution, as the calculations of chemical shifts show. For simplicity, *M1* ⇌ *M2* in solution may be approximated as the equilibrium *C1* ⇌ *C7* (Figure 6).

Related phenyl (ring D) and C-OH rotamers, which do not belong to groups i and ii, e.g., *C13* with an opposite orientation of the O-H bond of the 23-positioned hydroxyl group relatively to its orientation in *C1*, do not make substantial contributions to the equilibrium between the two preferred geometries of the piperideine−ethylenebiphynelene fragment. These rotamers show worse correlations and they are not considered in depth. Probably, the positioning of phenolic functions and the orientation of the O-H bonds make a great contribution to solvation energy. Then, upon the phenyl rotation or reorientation of the OH bonds, the energy changes are sufficient to discriminate a majority of rotamers that nevertheless share the same two favored geometries of piperideine−ethylenebiphynelene backbone.

c. Comparison of Δδ Values: Truncated Solvation Model. As discussed above, the implicit solvation model used in this study cannot be expected to yield satisfactory values of CCS, due to H-bonding between solvent and phenol rings of haouamines. In order to verify the conformer selection using this minimal model (Sections 2a and 2b), further calculations were performed modeling at least partial solvation of the OH groups.

There is no intramolecular H-bonding either in *C1* or in *C7* (Figure 6). Hence, OH moieties of phenol **1** are successful proton donors for H-bond formation. H-bonding between OH groups is stronger than between hydroxyl and keto groups.[17] It is reasonable to assume that phenolic hydroxyls of **1** are H-bonded with water molecules even in acetone solution that contains moisture. Therefore the solvate has been represented as a trihydrate (a truncated supermolecule; see Figure 7 for structure of *C1*·$3H_2O$). In this trihydrate, polycycle **1** binds three water molecules through one of its three hydroxyl groups (5-, 14- and 31-OH).

Concerning the correlation of "conformation-sensitive" chemical shifts, the standard error σ_r was found to be smaller for Δδ in trihydrates of *C1* and *C7* (Table 3) than for Δδ of the same atom pairs in the implicit continuum solvation model (see also Supporting Information for an increased accuracy for δ). The error is decreased by 0.2 and 0.4 ppm, respectively, for *C1* and *C7*. Conversely, the error is increased by 4.0 and 1.6 ppm for solvates *C9*·$3H_2O$ and *C10*·$3H_2O$, for which the errors reach intolerably high values, respectively, of 4.7 and 4.1 ppm (see Supporting Information for these structures). The superior trihydrate model consolidates the results of the continuum solvation model (Section 2b), identifying *C1* and *C7* as more "successful," and *C9* and *C10* as even less satisfactory fits to

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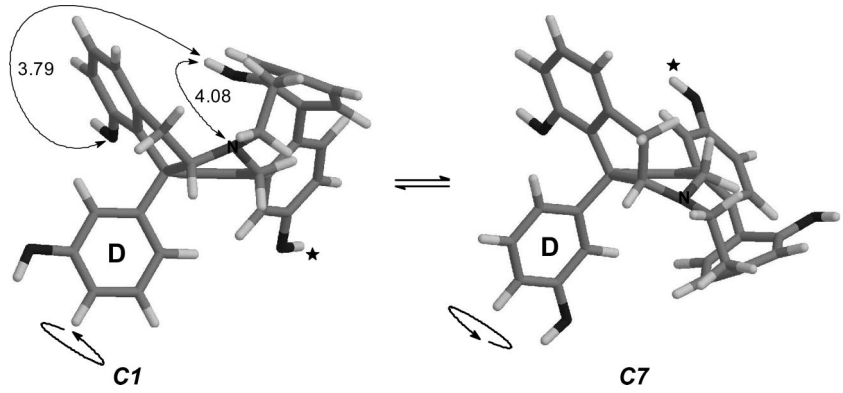

FIGURE 6. Equilibrating conformers *C1* and *C7* (one-headed arrows indicate free rotation of ring D, two-headed arrows show interatomic distances, Å). The N substituent is equatorial in both structures. These conformers show differences in the geometry of the tetrahydropyridine cycle, the rotational orientation of phynelene rings A and B and phenyl ring D, and the orientation of the O−H bond for the 5-positioned OH group (the corresponding H is labeled with an asterisk).

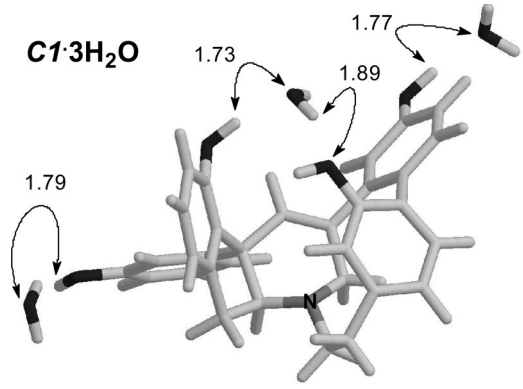

FIGURE 7. Optimized geometry of solvate *C1*·3H_2O and intermolecular O···H distances (Å).

TABLE 3. Experimental[1] ($\Delta\delta_{exp}$), Calculated ($\Delta\delta_{calc}$), and Standard Regression Error (σ_r) Values for Differences of ^{13}C NMR Chemical Shifts (ppm) for Selected Carbon Pairs of Conformers *C1*, *C7*, and *C10* of Solvated Alkaloid 1·3H_2O

entry	Δδ C-16/C-1	Δδ C-12/C-9	Δδ C-11/C-13	Δδ C-26/C-16	Δδ C-27/C-32	σ_r for *M1*	σ_r for *M2*
M1[a]	3.0	16.4	−4.0	6.5	32.7		
M2[a]	12.4	19.4	12.9	1.8	38.5		
C1·3H_2O	4.9	15.6	−1.4	7.3	34.9	0.7	8.0
C7·3H_2O	12.8	13.9	13.3	4.3	41.9	4.4	1.4
C9·3H_2O	4.3	10.4	−2.1	12.9	27.3	4.7	11.1
C10·3H_2O	14.2	12.3	14.0	6.1	41.6	10.4	4.1

the experimental data. Hence, conformers *C9* and *C10* do not contribute appreciably to the experimentally detected[1] equilibrium. It is important to note that conformers *C9* and *C10* are the must successful structures among nonhydrated ^{1}Sf and Sf_1 sofas that show a poor correlation for a nonhydrated molecule **1** (Table 2). Therefore the role of other conformers in the detectable equilibrium should be even lower. One may claim that equilibrium *C1* ⇌ *C7* (Figure 6) resulted from our CCS analysis is an accurate approximation of the observed[1] equilibrium *M1* ⇌ *M2*.

3. Conformational Dynamics in 1. Comparison of the geometry of any two conformers, e.g., favored conformers *C1* and *C7*, shows which intramolecular dynamic processes could be involved in their interconversion. However, the conformer structure provides no information about which process is of the lowest kinetic barrier among alternative dynamic processes, if they are present. Concerning stability of low and high energy conformers of polycycle **1**, the axial conformers are of quite different relative stability compared with the equatorial ones (see Section 1a for ΔE estimates). This fortunate circumstance makes it possible to suggest the lowest energy conformational pathway between *C1* and *C7* not estimating conformational barriers accurately and thereby to identify the intramolecular motions involved in the detected[1] conformational transformation. Below, all intramolecular motions in **1** will be considered, excluding a trivial rotation of the phenyl substituent (ring D; Figure 6).

a. NIR. From the viewpoint of conformation of vicinal substituents at the C−N bond, NIR in alkylamines is actually an interconversion, transforming one staggered conformation of these substituents into another.[8a,b,18a] In this case, NIR includes a 60° turn of N-alkyl substituents.[8a,b,18a] Substituent eclipsing is very rare in alkylamines, and if found, it occurs only in the lowest energy conformer.[19a–c] As a consequence, these amines undergo isolated N-inversion (INI) and not NIR; in other words, the N-substituent is not rotated upon inversion of the nitrogen pyramid.[18b,19b,c]

Amine **1** is the first studied alkylamine where both interconverting conformers, *C1* and *C7*, possess such an eclipsed geometry (Figure 8). The reason for the prevalence of the eclipsed conformers is clear: the ethylenebiphynelene bridge covalently locks its N-CH_2 fragment in *C1* and *C7* into what appears at first glance to be unfavored geometries. Compared with the case of one eclipsed conformer,[19a–c] one may say that the "doubled" eclipsing results in a NIR recovery. Interconversion of these conformers includes inversion of the nitrogen pyramid, which is accompanied by a 180° rotation around the C−N bond (Figure 8). Since the interconversion also incorporates RI, the overall motion of the N-substituent is of a greater magnitude than for NIR in common alkylamines. This motion of the $NCH_2CH_2C_6H_4C_6H_4$ bridge forces its constituent, the N-CH_2 fragment, to be rotated by a much larger angle than the canonical 60° for NIR.

Furthermore, also interconversion of eclipsed conformer and staggered conformer takes place for amine **1**, e.g., in transformations *C1* ⇌ *C2* or *C7* ⇌ *C8*. Orientation of vicinal

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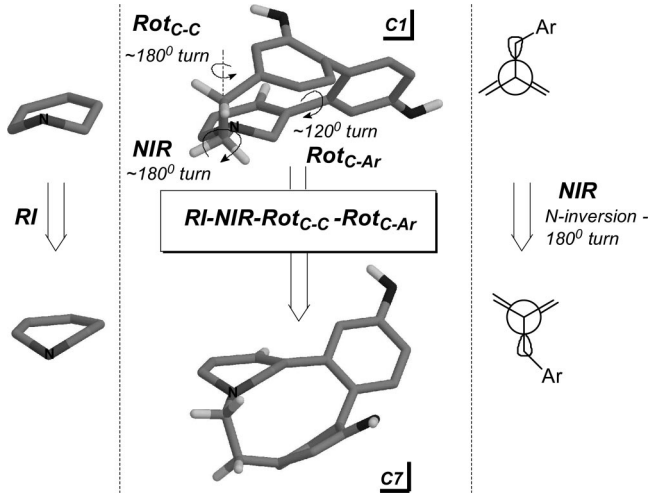

FIGURE 8. Conformational transformation *C1* → *C7* via a concerted RI-NIR-Rot$_{C-C}$-Rot$_{C-Ar}$ process (middle). Only the flexible tetrahydropyridine−*N*-ethylenebiphenylene fragment of the ab initio-optimized structures of *C1* and *C7* is shown for clarity. Left: RI for the tetrahydropyridine fragment. Right: NIR for the amino fragment.

substituents at the exocyclic C−N bond is synperiplanar (eclipsed) in equatorial conformers, e.g., *C1* and *C7*, whereas it is gauche (staggered) in axial conformers, e.g., *C2* and *C8* (Figures 2 and 3). However, the situation for amine stereodynamics in the case of one stable eclipsed conformation is reversed for amine **1**. In contrast to alkylamines with such an eclipsed conformation for an N-substituent,[19a–c] there is no INI in **1**: transformations *C1* ⇌ *C2* or *C7* ⇌ C8 involve N-inversion as well as a ∼60° rotation of the N-substituent. In other words, interconversion of these stereoisomeric forms does occur via NIR. This "extra" rotation is a consequence of covalent locking of the N-substituent in **1**. Inversion of the nitrogen pyramid leads to 3D reorganization of the entire $CH_2CH_2C_6H_4C_6H_4$ bridge, the motion of which also includes a C−N rotation. As the structures of N-invertomers *C1* and *C2* or *C3* and *C4* show (Figure 2), NIR additionally requires rotation around the CH_2-CH_2 bond of the *N*-ethylene chain.

Thus, conformational behavior of the amino fragment of **1** is unusual. There are stable eclipsed geometries as well as a "nonstandard" coupling of nitrogen inversion and C−N rotation. A reasonable suggestion for these anomalies is that they arise from a rigidity of the "building blocks" of the paracyclophane fragment. This fragment contains three rigid units (*para*-disubstituted phenylene ring B, *ortho*-disubstituted phenylene ring A, and the NCH_2C unit of the piperideine cycle) and only one unit with some rotational freedom (N-CH_2-CH_2-). Being closed into a shortly bridged paracyclophane, the rigid units cannot be arranged in the space satisfying rotational preferences of all vicinal substituents in this fragment. As a result, an eclipsed conformation appears in the flexible unit (for the exocyclic C−N bond) for a majority of conformers of **1**. Besides, phenylenes A and B are oriented perpendicularly (see Figure 6), destroying their conjugation completely.

b. RI-Associated Concerted Motions. RI of the tetrahydropyridine cycle of **1** does not proceed as an isolated process.[20] Any conformational change of the piperideine fragment causes a change in the geometry of the *N*-ethylenebiphenylene bridge; this is obviously because of severe constraints in the strained molecular structure. RI in **1**, when it takes place, is geared with NIR and other rotations.

Indeed, the performed conformational search (see Section 1) demonstrates that equatorial forms adopt only one of two 3D geometries of the flexible tetrahydropyridine−ethylenebiphenylene backbone: as it is in the in *C1* and C3 with the ^{1}Sf-shaped azacycle or as it is in C5 and *C7* with the Sf_1-shaped azacycle (Figures 2 and 3). In other words, the $NCH_2CH_2C_6H_4C_6H_4C$ chain is almost rigid in low-energy (i.e., equatorial) conformers; even rotation of ring B is questionable when there is no RI.[3] Only RI enables rotational freedom of the entire $NCH_2CH_2C_6H_4C_6H_4C$ chain (change of its torsional angles) in addition, as mentioned, to RI-independent interconversions *C1* ⇌ *C3* and *C5* ⇌ *C7*. It is transparent therefore that a direct transformation *C1* ⇌ *C7*, which includes changes of almost all torsional angles of this chain, occurs as a concerted process.

It combines several intramolecular motions (Figure 8): (i) RI (the ^{1}Sf → Sf_1 transformation), (ii) NIR (pyramidal inversion coupled with a 180° turn of the exocyclic N-substituent), (iii) a 180° rotation around the CH_2-CH_2 bond (Rot$_{C-C}$), and (iv) rotation of ring A around the C-2−C-3 bond (Rot$_{C-Ar}$) followed by a separate process, a phenyl rotation (ring D). On the other hand, phenylene rings A and B keep their relative orientation in the *C1* ⇌ *C7* transformation, i.e., phenylene B is not rotating when RI occurs.

c. Preference for Five Concerted Motions. Alternative pathways from *C1* to *C7* involve other conformers, e.g., *C1* ⇌ *C2* ⇌ *C15* ⇌ *C7*. These pathways pass through high-energy minima corresponding to axial conformers such as *C2*, *C4*, *C6*, or *C8* (Figure 2); their transformation to *C7* takes place via a triple process, RI-ISR-ISR. The direct *C1* ⇌ *C7* transformation via concerted RI-NIR-ISR-ISR is probably the lowest energy pathway, it corresponds to the experimentally observed[1] stereodynamics. Calculated Δ*E* for energy minima *C2* versus *C1* is 13.0 kcal/mol. In order for this alternative pathway to be favored, the sum of this Δ*E* and the kinetic barrier for RI-ISR-ISR should be lower than the barrier for the direct RI-NIR-ISR-ISR transformation. However, the amplitude of the RI-ISR-ISR intramolecular motion is even bigger than for RI-NIR-ISR-ISR. Therefore it is highly unlikely that the RI-ISR-ISR barrier is lower than that for RI-NIR-ISR-ISR by more than 13.0 kcal/mol. Thus, from the point of view of conformational dynamics of alkylamines,[18a,b] bridged cyclic alkylamine **1** is a unique system: bearing a strained bridge as an exocyclic N-substituent, it shows unprecedented stereodynamics of five concerted motions (NIR comprises two of them).

In summary, it is worth remarking that CCS-based conformational analysis is incomparably more reliable than analysis by calculations of relative energy. Energy calculations do not afford sufficient accuracy if a high level of ab initio theory is not used; so far such studies for molecules of ∼50 and more atoms are limited by resources of conventional computer clusters. In contrast, sufficiently accurate chemical shifts may be supplied by routine levels of DFT calculations (see Supporting Information for additional comments).

Using a set of experimental chemical shifts is a more accurate test for the quality of computational results than a "one value" comparison of experimental Δ*G* and calculated energy. Experimental data for relative stability of other conformers are absent for **1**. Generally speaking, such data are rarely available for multiconformation systems and experiments usually provide only one Δ*G* value, which is

(20) The opposite is incorrect: NIR and some rotations may be not coupled with RI (see Section 3a).

related to the two lowest energy structures. Therefore there is no possibility of performing statistical analysis of errors in energy calculations for a flexible system and thereby reaching valid estimates for relative stabilities of conformers of similar energies. In contrast, statistical analysis of *sets* of different chemical shifts for alternative structures provides a way to exclude systematic (constant) error. Moreover, this error is eliminated even if it is of arbitrarily large magnitude. As the example of polycycle **1** demonstrates, the remaining nonsystematic calculation errors still permit reliable identification of experimentally detected conformers without resorting to laborious energy calculations of high accuracy.

The CCS approach utilizes routine NMR spectra and therefore it may reduce the need for complicated NMR experiments on systems of known chemical connectivity and unknown stereochemistry. In light of the rapid progress of computerized research, conformational analysis by CCS merits consideration as a practical complement to NMR for conformational analysis of organic molecules, including large systems. Of course, a significant number of conformers, the presence of heavy atoms in analyzed structures and specific solvation effects are obvious limiting factors for this methodology.

Experimental Section

Crude geometries for molecular forms of **1** were obtained by means of molecular mechanics calculations using MM3* force fields (for nonionized structures) and OPLS force fields (ionized structures), as implemented in the Macromodel 6.5 package.[21a–c] The Monte-Carlo option was used to explore the conformational space of **1** (generation of 10^5 structures with the upper energy limit of 20 kcal/mol from the lowest energy conformer found). The no solvent, extended cutoff, and distance-dependent dielectric electrostatics options were employed for the energy minimization.

These geometries were used as starting structures for DFT calculations at the B3LYP/6-31G(d) level by means of the Jaguar4.0 package[22] for gas phase. Energy minimization was performed for these gas phase conformers using a self-consistent reaction field to approximate acetone solvent,[10b] as implemented in Jaguar. Geometries of ionized species of **1** were obtained by a formal transfer of one of the phenolic protons to the amino group in ab initio-optimized conformers followed by energy minimization at the B3LYP/6-31G(d) level for gas phase. This level of calculations served as an initial selector of low energy conformers: only structures with $\Delta E \leq 5$ kcal/mol relative to the conformer of the lowest B3LYP/6-31G(d)-determined energy were included in further computations. The geometry of resulting structures as well as solvates (see below) was reoptimized at the B3LYP/6-31G(d,p) level for acetone solution. The difference ΔE between total molecular energies was not corrected to the zero point energy excluding structures *C1* and *C7* (see Supporting Information). Preoptimized solvate geometries were generated placing the oxygen atom of the solvent molecule (water) at a 2 Å distance from the proton of the OH group(-s) of optimized structures of conformers *C1*, *C7*, or *C10*. The axis of the O−H bond and the C_2 symmetry axis of the water molecule were arranged to be approximately coaxial in these starting structures.

^{13}C chemical shifts were calculated at the B3LYP/6-31+G(d) level using the GIAO utility[10c-e] of the Gaussian98 package[23] for the final, B3LYP/6-31G(d,p)-derived structures with $\Delta E \leq$ 4 kcal/mol relative to the global minimum as well as for the betain *C17*. Regression analysis for experimental δ_{exp} as well as $\Delta\delta_{exp}$ and calculated δ_{calc} as well as $\Delta\delta_{calc}$ values was undertaken for each pair of structures C_i/*M1* as well as C_i/*M2*; standard error of regression σ_r was used to select structures C_i providing the best correlation (see Supporting Information for methodological notes).

Acknowledgment. This research is supported by the governmental KAMEA program. I am very grateful to a group of professors from the Israeli universities (Profs. Benjamin Fain, Eliezer Giladi, Dan Amir, Daniel Hupert, Elisha Haas, and others) who have initiated this program and enthusiastically supported its ratification.

Supporting Information Available: Rotation-related discussion and methodological notes; optimized geometry (Cartesian coordinates), calculated chemical shifts, and the corresponding rms values for selected conformers. This material is available free of charge via the Internet at http://pubs.acs.org.

JO702766X

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